American Journal of Pathology, Vol. 147, No. 1, July 1995

Accumulation of Extracellular Matrix and Developmental Dysregulation in the Pancreas by Transgenic Production of Transforming Growth Factor-β1

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Transgenic mice expressing transforming growth factor-β1 (TGF-β1) in the pancreatic β-islet cells directed by human insulin promoter were produced to study* in vivo *effects of TGF-β1. Fibroblast proliferation and abnormal deposition of extracellular matrix were observed from birth onward, finally replacing almost all the exocrine pancreas. Cellular infiltrates comprising macrophages and neutrophils were also observed. Plasminogen activator inhibitor was induced in the transgenic pancreas as well as fibronectin and laminin, partly explaining accumulation of extracellular matrix. TGF-β1 inhibited proliferation of acinar cells* in vivo *as evidenced by decreased bromodeoxyuridine incorporation. Development of pancreatic islets was dysregulated, resulting in small islet cell clusters without formation of normal adult islets; however, the overall islet cell mass was not significantly diminished. Additional transgenic lines with less pronounced phenotypes had less expression of TGF-β1 transgene. These findings suggest that TGF-β1 might be a mediator of diseases associated with extracellular matrix deposition such as chronic pancreatitis, and this mouse model will be useful for further analysis of the* in vivo *effects of TGF-β1, including its potential for immunosuppression. (Am J Pathol 1995, 147:42–52)

Transforming growth factor-β (TGF-β) is involved in such diverse physiological processes as cell proliferation, embryogenesis, extracellular matrix (ECM) formation, and wound healing.[1] TGF-β affects cell replication by either promoting or inhibiting the cell cycle according to the type of cell involved or the presence of other growth factors *in vitro*.[2] This cytokine can also influence immune responses by augmenting the migration of monocytes and their production of cytokines[3,4] or by inhibiting several T and B lymphocyte functions *in vitro*.[5,6]

Like many cytokines, TGF-β yields a variety of effects *in vivo*. For example, infusion of human TGF-β into the synovial space or subcutis induced neutrophil recruitment.[7,8] On the other hand, systemic administration of TGF-β protected the recipients from experimental allergic encephalomyelitis and collagen-induced arthritis.[9,10] Additionally, anti-proliferative effects have been demonstrated in transgenic mice whose targeted expression of TGF-β inhibited skin or mammary gland development.[11,12] However, definitive fibrosis was not observed in those mice, although such a change could be anticipated from *in vitro* experiments. The expression of TGF-β-inducible proteins was also not studied well *in vivo*, which could be important in elucidating the mechanism of TGF-β action.

We produced transgenic mice expressing TGF-β1 in the pancreatic β-islet cells, and analyzed the resulting phenotypes including the

M. Lee was supported by a fellowship from the Multiple Sclerosis Society (FA 1007-A-1), and D. Gu was supported by a fellowship from the Juvenile Diabetes Foundation. This work was supported by grants from the National Institutes of Health (grants HD29764 and HL48726 to N. Sarvetnick, and DK20043 to C. B. Wilson). This is manuscript number 8999-NP from The Scripps Research Institute.

Accepted for publication April 3, 1995.

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expression pattern of proteins that could be related to the phenotypes. We observed fibrosis, infiltration of monocytes and neutrophils, developmental dysregulation, induction of ECM proteins and plasminogen activator inhibitor (PAI), and some structural perturbations in the pancreatic tissue of these mice.

Materials and Methods

Production of Transgenic Mice

The DNA plasmid containing mutated porcine TGF-β1 with serine residues at amino acid positions 223 and 225, which enables secretion of active molecules without acid treatment, was cut with *Bgl*II.[13,14] This sequence was inserted into the *Bam*HI site of a plasmid containing the human insulin promoter and a terminator sequence from hepatitis B virus. The fragment cut with *Eco*RI and *Sph*I was isolated and microinjected into the transgenic founders (Ins-TGF-β1 mice) (Figure 1).

Histopathological studies of the transgenic mice were done by using hematoxylin and eosin (H&E) or Gomori's trichrome staining of pancreatic sections after fixation in zinc formalin (Anatech, Battle Creek, MI) and paraffin embedding. Blood glucose of the mice was measured with a Glucometer 3 (Miles Inc., Elkhart, IN).

Transgene Typing

The presence of transgene in the founders and their progeny was confirmed by polymerase chain reaction typing of their tail DNA using primers specific for human insulin promoter sequences (CCTGGTCTAATGTGGAAAGTG and TGCAATTTCCGGACCATTTCC).

Immunohistochemical Staining

Deparaffinized sections of the Bouin-fixed pancreas were stained by using an immunoperoxidase method.

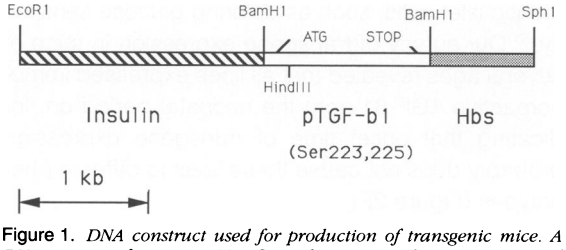

Figure 1. *DNA construct used for production of transgenic mice. A DNA sequence for porcine TGF-β1 with serine residues at amino acid positions 223 and 225 was inserted into the* Bam*HI site of a plasmid containing the human insulin promoter and a hepatitis B virus terminator sequence.*

Primary antibodies used for these sections were polyclonal antibodies to porcine TGF-β1 (Wyss-Coray T, Feng L, Masliah E, Ruppe MD, Lee HS, Toggas SM, Rockenstein EM, Mucke L, Am J Pathol, in press), porcine insulin (DAKO, Carpinteria, CA), synthetic glucagon (Chemicon, Temecula, CA), human somatostatin (DAKO), human pancreatic polypeptide (ICN, Lisle, IL), human amylase (Sigma Chemical Co., St. Louis, MO), human fibronectin (FN) (Sigma Chemical Co.), mouse laminin (LM) (Sigma Chemical Co.) or rat PAI (see below). Incubation with specific biotinylated secondary antibody and avidin-biotin-peroxidase complex (Vector, Burlingame, CA) followed. After color reaction with diaminobenzidine (Sigma Chemical Co.), the sections were counterstained in hematoxylin or methyl green, and observed after dehydration in graded alcohol. Fresh-frozen sections of the pancreas were also stained with monoclonal antibody to CD4 (PharMingen, San Diego, CA), CD8 (PharMingen), B220 (PharMingen), Mac-1 (Boehringer-Mannheim, Indianapolis, IN), and α-chain of VLA-4 (kindly provided by Irving L. Weissman, Stanford University Medical School, Stanford, CA), or F4/80 (Serotec, Oxford, UK) after acetone fixation.

Production of Anti-PAI Antiserum

A polyclonal antibody against rat PAI was raised in rabbits according to a method previously described.[15] The *Pst*I/*Stu*I fragment of rat PAI cDNA was blunt-ended and subcloned into the *Eco*RV site of pBluescript. The *Xba*I/*Xho*I fragment was subcloned into pGEM1 to produce pETPAI-1. BL21 (DE3) bacteria transformed with pETPAI-1 were cultured, and proteins were purified from the culture using Ni-NTA affinity resin (Qiagen, Chatsworth, CA). A polyclonal antiserum was raised by immunizing rabbits with the recombinant protein. An initial dose of 1 mg of the protein in complete Freund's adjuvant was injected subcutaneously, followed by four weekly immunizations with 0.5 mg protein in incomplete Freund's adjuvant.

RNAse Protection Assay

To verify expression of the TGF-β1 transgene at the RNA level, an RNAse protection assay was done using a porcine TGF-β1-specific riboprobe according to a previously described method with some modifications.[16] Briefly, pancreatic RNAs from transgenic mice and their nontransgenic littermates were prepared by homogenization in 5 mol/L guanidium thiocyanate solution followed by acid-phenol extraction.

A recombinant plasmid containing a 253 bp fragment of porcine TGF-β1 and one containing a 90 bp fragment of mouse β-actin were linearized and transcribed by appropriate RNA polymerase with incorporation of ^{32}P-UTP. Fifty μg of total pancreatic RNA was precipitated and dissolved in hybridization buffer containing 1×10^5 cpm anti-sense riboprobe. After overnight hybridization, unhybridized RNA was degraded with RNAse T1 and RNAse A (Sigma Chemical Co.). After the addition of Stop Buffer (Continental Scientific, Inc., San Diego, CA) and isopropanol precipitation, the samples were electrophoresed on a 6% polyacrylamide gel, dried, and scanned on an AMBIS system (AMBIS Inc., San Diego, CA). The data were presented as a radioactivity ratio of specific mRNA to β-actin mRNA signal.

Production of TGF-β1 by Transgenic Pancreata

Adult islets were isolated as previously described.[17] Isolated islets were cultured for 5 days in RPMI 1640 supplemented with 20 mmol/L Hepes, 300 μg/ml L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and either 10% fetal calf serum (FCS) or 1% Nutridoma-SP (Boehringer-Mannheim). To measure TGF-β1 production by neonatal transgenic pancreata, five transgene-positive neonatal pancreata were digested with collagenase P (Boehringer-Mannheim). Whole digest was cultured in RPMI 1640 supplemented with 20 mmol/L Hepes, 300 μg/ml L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS. TGF-β1 levels in the culture supernatants of adult islets or whole neonatal pancreatic digest were measured with a modified bioassay method utilizing a PAI-1 promoter-luciferase construct.[18] In brief, human hepatoma cells (Hep3B) were stably transfected with an expression vector containing a truncated PAI promoter (1.5 kb) fused to the firefly luciferase reporter gene. After 24 hours of incubation with test samples, the cell extract was assayed for luciferase activity as previously described.[19]

BrdU Staining

Bromodeoxyuridine (BrdU) (Serva, Heidelberg, Germany), 100 μg/g, was injected intraperitoneally into mice 12 hours before sacrifice. Paraffin-embedded sections of their dissected pancreata were stained with an anti-BrdU antibody (Accurate Chemical, Westbury, NY) after treatment with 2.8 N HCl for 15 minutes. Mitotic index was calculated by dividing the number of stained nuclei with that of total nuclei of more than 2000 cells in five randomly chosen, high-magnification fields in each pancreas.

Statistical Analysis

Student's *t*-test was employed to compare pancreatic weight and mitotic indices between transgenic and nontransgenic groups.

Results

Characterization of Transgene Expression and the Resulting Phenotype

Our microinjection procedures yielded 10 male transgene-positive founders (four females were not analyzed further). Examination of the pancreatic histopathology of these lines at the age of 4 months resulted in their division into three categories: the first was a "severe" phenotype (S line) in which five founder lines exhibited replacement of most exocrine tissue by ECM (Figure 2A). Second, a "moderate" phenotype (M line) contained another two lines whose islets had a somewhat disorganized appearance but exocrine pancreata had minimal ECM deposition (Figure 2B). The third, or "normal" phenotype (N line), contained another three founders that exhibited no gross abnormality in their pancreata (Figure 2C). We chose one representative line from each founder category for detailed analysis.

First, we studied the expression of TGF-β1 in these three transgenic lines at the protein level. Immunohistochemistry demonstrated that pancreatic islets from all three (S, M and N) lines harbored islet-specific TGF-β1 protein. The staining pattern revealed a notable nonequivalence of transgene expression among cells within the islets in all three lines. That is, TGF-β1 immunoreactivity varied from high to low or undetectable within individual islets (Figure 2, C–E). This variation could reflect heterogeneity among pancreatic islet cells, such as differing glucose sensitivity.[20] Our survey of transgene expression in mice of several ages revealed that all lines expressed immunoreactive TGF-β1 from the neonatal period on, indicating that onset time of transgene expression probably does not cause these lines to differ in phenotypes (Figure 2F).

We next studied the gross and histopathological manifestations of TGF-β1 expression in our transgenic mice. Morphologically, mice from the S line underwent dramatic disruption of the normal pancreatic

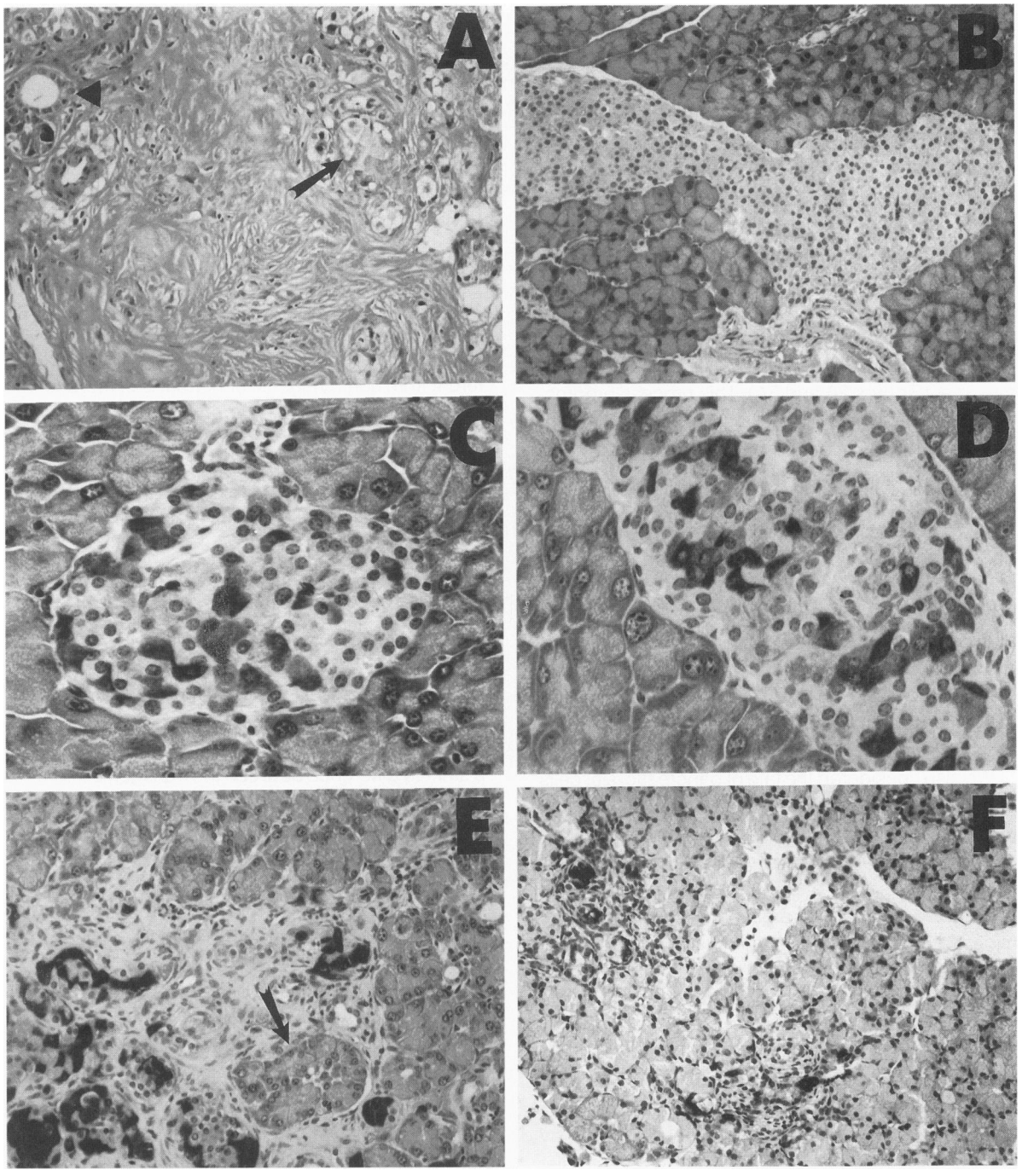

Figure 2. (A) *Gomori's trichrome staining of the pancreas from a 4-month-old S line transgenic mouse. Almost all of the exocrine pancreas was replaced by abnormal ECM deposition. Small round islet cell clusters* (arrow) *and ductal metaplasia* (arrowhead) *were also observed.* (×200) (B) *H&E staining of a pancreatic section from a 4-month-old M line mouse. Islets showed swollen and irregular morphology; however, abnormal deposition of ECM was almost absent.* (×200) (C) *Anti-TGF-β1 staining of a pancreatic section from a 4-month-old N line mouse. No gross histological abnormality was observed. Some cells expressed TGF-β1 strongly or weakly, but other cells did not.* (×400) (D) *Anti-TGF-β1 staining of a pancreatic section from a 4-month-old M line mouse. Nonequivalence of transgene expression was noted in this line also.* (×400) (E) *Anti-TGF-β1 staining of a pancreatic section from a 6-week-old S line mouse. Transgene expression varied within individual islet, as in the other two lines. Abnormal deposition of ECM progressively replaced the exocrine pancreas and led to fragmentation of exocrine pancreas* (arrow). *Infiltration of mononuclear cells and neutrophils was also noted.* (×200) (F) *Anti-TGF-β1 staining of a neonatal pancreatic section from an S line mouse. TGF-β1 expression was observed in the neonatal pancreas of M and N line as well. Fibroblast proliferation was observed in the peri-islet region.* (×200)

architecture. The pancreas was small and firm, particularly in adult mice more than 4 months of age. The average weight of the pancreas of the S line mice more than 4 months of age ($n = 7$) was 73.6 ± 10.6 mg (mean ± SD), being significantly lower than that of nontransgenic mice of the similar age (178.3 ± 16.2 mg) ($n = 7$, mean ± SD) ($P < 0.001$). Overt microscopic changes began during the neonatal period when the peri-islet region was infiltrated by fibroblasts (Figure 2F). Abnormal deposition of ECM was demonstrable from birth onward, and definitive accumulation of ECM developed around islets between 2 and 4 weeks of age as demonstrated by Gomori's trichrome staining that shows the existence of collagen fiber (Figures 2A and 3A). This process progressively replaced the exocrine regions, leading to fragmentation of the exocrine pancreas (Figures 2E and 3A). In adult animals older than 4 months, almost all exocrine areas were replaced by ECM, leaving only small remnants of acinar cell clusters. These fibrotic pancreata were surrounded by adipose tissue. Immunohistochemical detection with anti-amylase antibody confirmed the progressive loss and final disappearance of exocrine tissue in mice older than 4 months of age (data not shown). Despite the near-complete loss of exocrine tissue, no signs of wasting or overt changes in feces were observed in adult mice of the S line.

We also considered the fate of pancreatic islet cells in our S line transgenic mice. Between the neonatal period and adult age of 4 months, the islet cells became progressively surrounded by fibroblasts and ECM (Figure 2, A, E, and F). In addition, these islets never attained a normal architecture typical of adult mice but instead were present in small cell clusters separated by ECM (Figures 2, A and E, and 4A). This observation suggests that development of pancreatic islets was dysregulated rather than that islets were fragmented by infiltrating ECM deposition.

Despite developmental abnormality of pancreatic islets, the islet mass did not appear to be significantly diminished from that in normal mice, as judged by anti-insulin immunostaining (Figure 4A). These islet fragments contained glucagon-, somatostatin-, or pancreatic polypeptide-positive cells in apparently normal numbers and distribution (Figure 4B). Despite these dramatic morphological changes in mice of the S line, they did not exhibit hyperglycemia during the 6-month observation period (<230 mg/dl) and were indistinguishable in viability and overall health from their nontransgenic littermates. The apparently normal islet function was consistent with the preserved islet cell mass in this line.

In addition to exocrine and endocrine changes, we observed endothelial hyperplasia, beginning at 2 to 4 weeks of age around pancreatic islets. Ductal metaplasia, often trapped in deposited ECM, were also noted occasionally (Figure 2A).

In M and N lines, no overt exocrine pathology was apparent during the 6-month observation period. Immunohistochemistry revealed normal distributions of insulin, glucagon, somatostatin, and pancreatic polypeptide in the pancreatic islets from both lines (data not shown). However, islet morphology was subtly abnormal in the M line in which islets exhibited swollen or irregular shape and were poorly circumscribed (Figure 2, B and D), whereas it was not different from nontransgenic or normal islet morphology in the N line (Figure 2C). In comparison, no cellular infiltration or abnormal accumulation of ECM was apparent in either the M or N lines of transgenic mice throughout the observation period. Transgenic mice from these two lines did not exhibit hyperglycemia.

Transgene Expression at RNA Level

We performed RNAse protection assays to document the presence of the transgene-specific transcript and to study the quantitative relationship between the transgene expression and phenotypes, which immunohistochemistry does not reveal. The results showed a striking difference between the transgenic lines: the radioactivity ratio between porcine TGF-β1 and β-actin signal was 0.0, 0.09, 0.16, and 0.33 in a nontransgenic littermate, an N line mouse, M line mouse, and S line mouse, respectively (Figure 5). In other words, the relationship between amount of TGF-β1 produced and phenotypes was clearly direct, since transgenic mice that produced the largest amount of TGF-β1 had the severe S phenotype, those that produced an intermediate level of TGF-β1 had the moderate M phenotype, and those that produced the smallest amount of TGF-β1 showed no phenotypic change (the N line).

Production of TGF-β1 by Transgenic Pancreata

We tried to measure bioactive TGF-β1 produced by the transgenic pancreata at the protein level. However, TGF-β1 was not detectable in the supernatant after 5 days of culture of adult pancreatic islets from all three lines. This experiment was repeated with a serum supplement instead of FCS because components of FCS might bind to TGF-β and inhibit the assay. However, TGF-β1 was still undetectable after 5

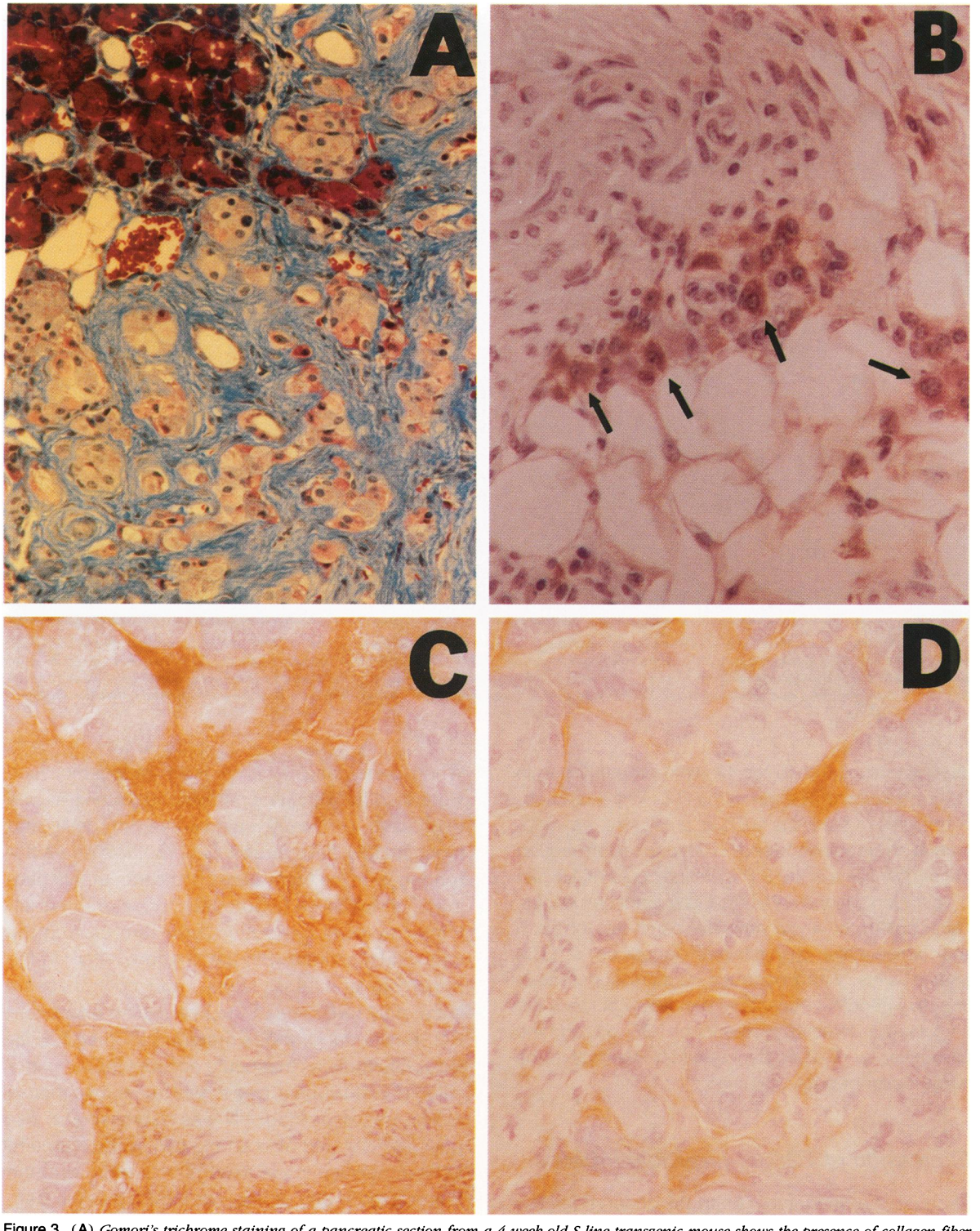

Figure 3. (A) *Gomori's trichrome staining of a pancreatic section from a 4-week-old S line transgenic mouse shows the presence of collagen fiber* (blue) *in the deposited ECM. Exocrine pancreas* (red) *was fragmented and replaced by ECM. Small islet cell clusters* (pink) *were scattered between ECM. (×200)* (B) *Anti-PAI staining of a pancreatic section from a 4-week-old S line mouse. PAI was expressed on spindle-shaped cells and mononuclear cells* (arrows). *(×400)* (C) *Anti-FN staining of a pancreatic section from a 2-week-old S line mouse. FN was expressed on deposited ECM and also on the cell surface around ECM deposition (×400)* (D) *Anti-LM staining of the adjacent section from the same 2-week-old S line mouse. In contrast to FN, LM was induced on the cell surface around ECM deposition but not on ECM. (×400)*

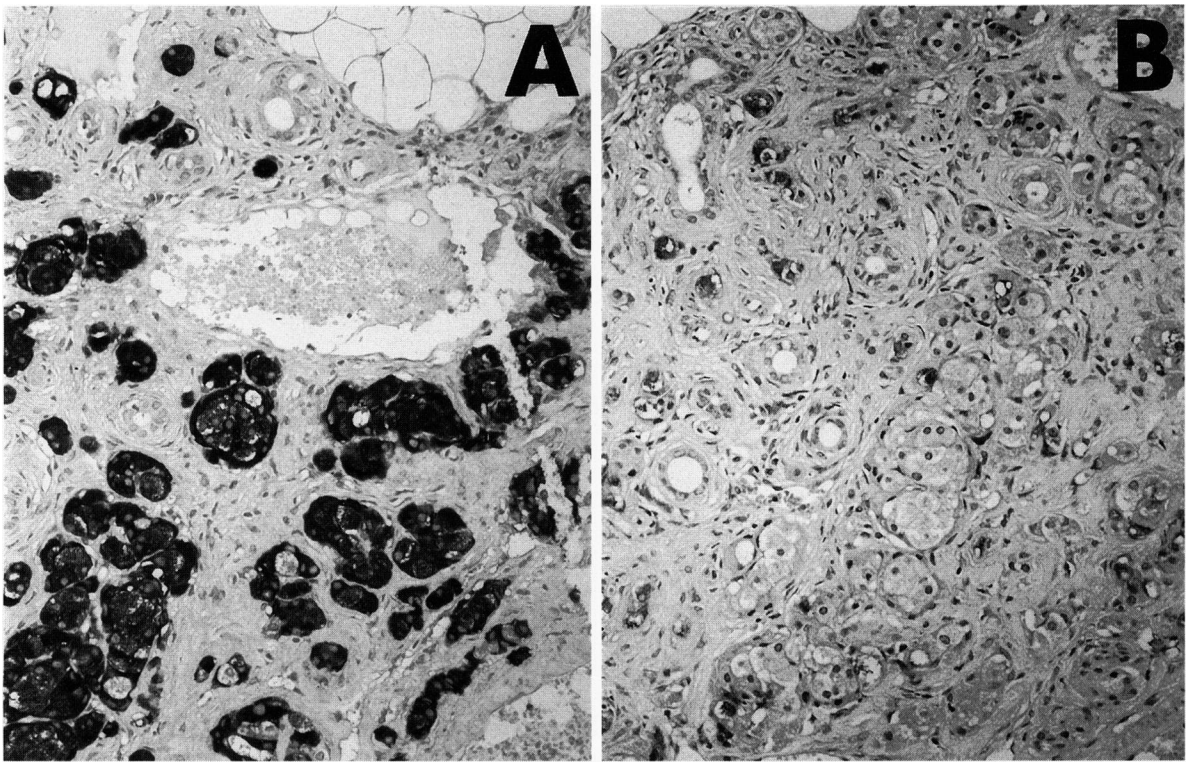

Figure 4. (A) *Anti-insulin staining of a pancreatic section from a 4-month-old S line mouse. Almost all exocrine pancreas was replaced by ECM; however, islet cell mass was not significantly diminished, although islets did not have normal architecture typical of adult mice.* (×200) (B) *Anti-glucagon staining of a pancreatic section from the 4-month-old S line mouse. Abnormal islet cell clusters contained glucagon-positive cells in apparently normal numbers and distribution.* (×200) *Somatostatin and pancreatic polypeptide-positive cells were also observed (data not shown).*

days of culture. These results were probably due to low production of TGF-β1 in the N or M line, and abnormalities of the islets such as small size, developmental dysregulation, and ECM deposition around transgenic islets in the S line, which could make islet preparation difficult and inhibit islet functions *in vitro*. Thus, culture of whole neonatal pancreata of the S line, which have only minimal deposition of ECM around islets, was performed. Culture supernatant from five neonatal transgenic pancreata of the S line contained a detectable amount of TGF- β1, and the production of 91.2 pg/pancreas/day was within the range described as functional.[21]

Expression of TGF-β1-Inducible Proteins

Because the phenotypic changes observed in our transgenic pancreata indicated to us that biologically relevant effects of TGF-β1 were present, we next sought the expression of the protein(s) that could mediate its actions. PAI can be induced by TGF-β *in vitro*,[22,23] and is important for the regulation of ECM degradation or angiogenesis.[24,25] When pancreatic sections from transgenic mice were studied with immunohistochemistry, PAI was expressed on many spindle-shaped cells and mononuclear cells, probably fibroblasts and macrophages, in the fibrotic area of the transgenic pancreas of the S line (Figure 3B). In the area not affected by ECM deposition, only rare cells in the interstitium stained with the antibody. Also, in the pancreata of M line, N line, and nontransgenic mice, only rare cells in the interstitium expressed PAI. Besides PAI, the expression of other TGF-β-inducible proteins that could account for the deposition of ECM substances in this transgenic mouse model was examined. FN was expressed on deposited ECM and also on the cell surface around ECM deposition in the transgenic pancreas of the S line (Figure 3C). It was not induced in the pancreas of nontransgenic mice or transgenic mice of the M or N line. In contrast, LM was induced on the cell surface around ECM deposition in the transgenic pancreas of the S line but not on ECM (Figure 3D). It was not induced in nontransgenic pancreas or transgenic pancreas of M or N line. Staining with

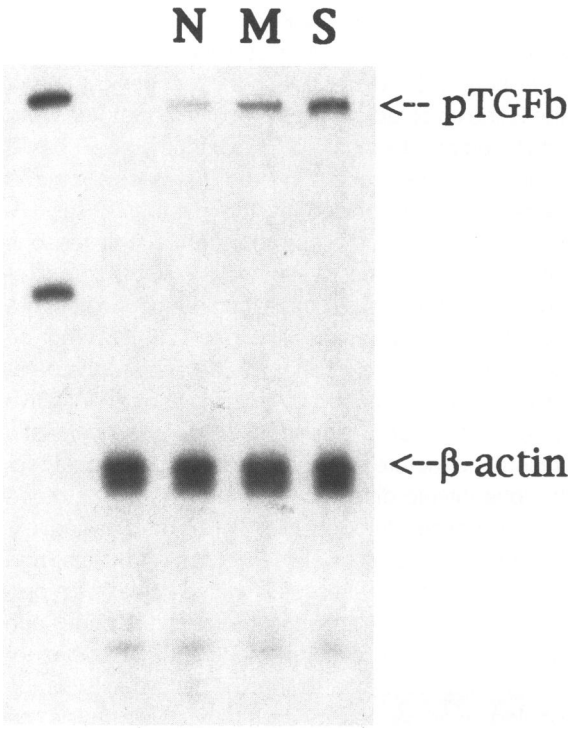

Figure 5. *RNAse protection assay of mRNA for porcine TGF-β1 and mouse β-actin. Fifty μg of total RNA was hybridized with riboprobes. The gel was scanned on AMBIS. Transgenic mice of severe phenotype (S line) shows the strongest TGF-β1 transcript signal, and transgenic mice with no abnormal phenotype (N line) shows the weakest signal, indicating the difference in the phenotypes between the lines are related to the amount of transgenic protein produced. In this assay, the size of the protected TGF-β1 and β-actin was shorter than that of the respective probes because the unhybridized polylinker regions in the riboprobes were digested by RNAse.*

control antibodies demonstrated the specificity of the PAI, FN, and LM staining on the pancreatic sections from the S line mice.

Characterization of Infiltrating Cells

Modest infiltration of mononuclear cells and neutrophils was observed around transgenic islets of the S line with H&E staining (Figure 2E). The inflammatory cell infiltration began at the age of 2 weeks, became prominent both in the areas affected by ECM deposition and within intact exocrine regions by the age of 4 weeks, and then appeared less severe as ECM replaced most of the exocrine tissue. These infiltrating cells were characterized by immunohistochemistry. Some cells stained with F4/80 antibody, indicating the presence of macrophages (Figure 6A). Staining with anti-Mac-1 antibody also demonstrated positive cells that comprise both macrophages and neutrophils present in the transgenic pancreas of S line (Figure 6B). To examine the expression of FN receptors on

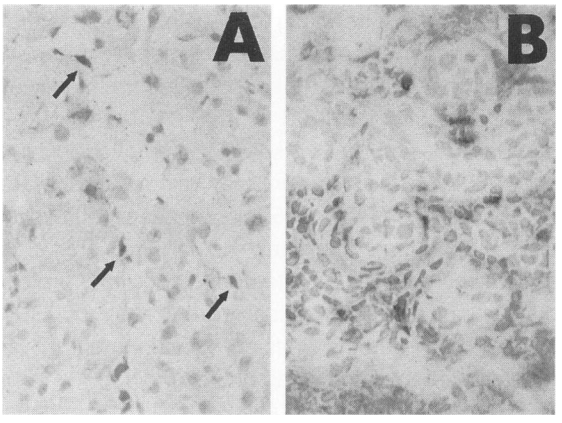

Figure 6. (A) *F4/80 immunostaining of a pancreatic section from a 6-week-old S line mouse revealed the presence of macrophages* (arrows). (×400) (B) *Anti-Mac-1 immunostaining of the same transgenic pancreas showed positive cells comprising both macrophages and neutrophils.*

infiltrating cells, pancreatic sections were also stained with an anti-VLA-4 antibody. Many infiltrating cells were positive for this FN receptor (data not shown). However, T or B lymphocytes positive for CD4, CD8, or B220 antigen were not observed. No cellular infiltration was observed in the transgenic pancreas of M or N line mouse.

Effect of TGF-β1 on Cell Proliferation

To investigate the effect of localized TGF-β1 on the proliferation of cells, we injected BrdU into 2-week-old transgenic mice and nontransgenic controls of the S line and measured its incorporation by their pancreata. The resulting mitotic indices of pancreatic exocrine cells from three individual transgenic mice were 1.7, 0.8, and 0.5% (1.0 ± 0.6%, mean ± SD), which was significantly lower than those of their nontransgenic littermates, ie, 10.0, 6.0, and 4.7% (6.9 ± 2.8%) ($P < 0.05$). However, the mitotic indices of 3-week-old M line transgenic (2.8 ± 0.7%, $n = 3$) and nontransgenic mice (3.2 ± 0.7, $n = 3$) were not different ($P > 0.1$).

Discussion

Studies of the phenotypes of the transgenic lines expressing variable amounts of TGF-β1 enabled us to demonstrate that the more TGF-β1 produced, the greater were pathological changes.

Three distinct transgenic lines were produced, each defined by the severity of the phenotypic changes observed in the pancreas. These were the

S, M, and N lines, denoting respectively severe structural changes in the pancreas, moderate changes, and no change. These phenotypes correlated well with the expression level of TGF-β1 transgene as demonstrated by an RNAse protection assay, which showed the relative amount of porcine TGF-β1 transcript in each phenotype. Efforts to measure the level of TGF-β1 in the supernatants of cultured islets with a bioassay and to correlate phenotypes with protein production were unsuccessful because of ECM deposition around the islets and developmental dysregulation of the islets in adult S line mice. However, TGF-β1 was detectable and within the functional range in the culture supernatant of total neonatal pancreata of the S line, which did not have much ECM deposition and did not require separation of individual islets. The onset of TGF-β1 expression was almost identical in three lines because the representative neonates from all lines expressed TGF-β1.

Fibroblast proliferation and ECM deposition were observed in the transgenic pancreata of S line mice, as anticipated from former *in vitro* studies.[26–28] However, mice expressing TGF-β1 in the skin or mammary glands did not manifest a clearcut response of this type.[11,12,29] On the other hand, transgenic mice expressing TGF-α, a cytokine belonging to a different family of growth factors, showed similar interstitial fibrosis.[30,31] However, other features were different as exemplified by absence of infiltrating cells and much more florid ductal changes in TGF-α transgenic mice. The mechanism by which different growth factors induce similar changes in the pancreas is not clear but might involve induction of common second mediator molecules such as PAI.

The expression of the proteins that constitute ECM and could be induced by TGF-β was also not clearly demonstrated in transgenic mice or after *in vivo* administration. We observed induction of FN and LM, important constituents of ECM; however, the expression pattern was different. FN was expressed on the cell surface and also on deposited ECM, which probably represent "cell surface FN" and "matrix FN," respectively.[32] On the other hand, LM was visualized only on the cell surface around ECM deposition, which indicates selective location of LM in the basal lamina. ECM deposition could have been mediated by increased synthesis of ECM substances[7] and also by induction of PAI, which we observed in our transgenic animals of the S line; PAI might then accumulate and could inhibit matrix degradation.

Induction of FN might also be related to the cellular infiltration observed in the transgenic mice of the S line. FN has been reported to mediate neutrophil chemotaxis in response to TGF-β through interaction with integrins such as VLA-4 or VLA-5 on neutrophils.[33]

Abnormal ECM deposition in transgenic mice of the S line involved mainly the exocrine pancreas and finally replaced almost all the exocrine area. The islet cells looked fragmented, but the islet mass appeared to be intact as judged by the number of insulin-positive cells and the normoglycemia. The reasons why exocrine tissue is affected by TGF-β1 and resulting ECM deposition more than endocrine cells are not clear. Cellular susceptibility to TGF-β1 might be different between exocrine cells and endocrine cells, which might be caused by qualitative or quantitative difference in TGF-β receptors. Instead, characteristic vascular structure in the pancreas might account for the phenotypic difference between them. The blood supply passes through islet cells first allowing exchange of materials between blood and islets, and then moves to acinar tissue,[34] which can explain pronounced effect of transgenic protein production such as TGF-β1 on the exocrine pancreas. Our developmental analysis indicated that the disturbed islet morphology was caused by a primary defect in islet organization as opposed to the disruption of already intact islet structures by ECM deposition. Possibly, changes in the expression of integrins or other adhesion molecules on islet cells that are critical for cell-to-cell or cell-to-ECM interactions during development might inhibit the formation of islet structure.[35]

Replacement of acinar tissue by ECM is probably not the only mechanism causing the disappearance of exocrine tissue. In our model, the transgenic pancreas incorporated significantly less BrdU in the exocrine tissue than the nontransgenic one, suggesting that acinar cell growth itself was affected by TGF-β1. This inhibition of growth may be mediated by blockage of cyclin-dependent protein kinases by TGF-β, as others found *in vitro*.[36] Thus, both impaired proliferation of acinar cells and ongoing ECM deposition probably contributed to the disappearance of exocrine tissue. However, apoptosis probably did not contribute to the disappearance of exocrine tissue in the S line because the number of free 3′-OH DNA ends was not increased in the nuclei of the exocrine pancreas as measured by terminal deoxynucleotidyl transferase-mediated addition of digoxigenin-nucleotides and anti-digoxigenin antibody staining (data not shown).

In conclusion, we observed ECM deposition, cellular infiltration, inhibition of proliferation, and developmental dysregulation of pancreatic islets in transgenic mice with pancreatic expression of TGF-β1. These findings support previous suggestions that TGF-β1 might be an important mediator of a variety of diseases associated with ECM deposition such as

pulmonary fibrosis, systemic sclerosis, or glomerulosclerosis of the kidney.[37] This mouse model will be useful for further detailed analysis of *in vivo* effects of TGF-β1 because almost all possible phenotypes expected from *in vitro* experiments were observed in these transgenic mice. This model may also serve as an animal model of chronic pancreatitis that has histopathological findings similar to the phenotypes of the S line such as fibrosis mainly affecting exocrine tissue and preservation of islet cells of all major types.[38,39]

References

1. Massague J: The transforming growth factor-β family. Annu Rev Cell Biol 1990, 6:597–641
2. Lyons RM, Moses HL: Transforming growth factors and regulation of cell proliferation. Eur J Biochem 1990, 187:467–473
3. Wahl SM, Hunt DA, Wakefield LM, McCartney-Francis N, Wahl LM, Roberts AB, Sporn MB: Transforming growth factor (TGF-β) induces monocyte chemotaxis and growth factor production. Proc Natl Acad Sci USA 1987, 84:5788–5792
4. Wahl SM: Transforming growth factor β (TGF-β) in inflammation: a cause and a cure. J Clin Immunol 1992, 12:61–74
5. Kehrl JH, Wakefield LM, Roberts AB, Jakewlew SB, Alvarez-Mon M, Derynck R BSM, Fauci AS: Production of transforming growth factor β by human T lymphocytes and its potential role in the regulation of T cell growth. J Exp Med 1986, 163:1037–1050
6. Wahl SM, Hunt DA, Wong HL, Dougherty S, McCartney-Francis N, Wahl LM, Ellingsworth L, Schmidt JA, Hall G, Roberts AB, Sporn MB: Transforming growth factor-β is a potent immunosuppressive agent that inhibits IL-1 dependent lymphocyte proliferation. J Immunol 1988, 40:3026–3032
7. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH, Fauci AS: Transforming growth factor type β: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. Proc Natl Acad Sci USA 1986, 83:4167–4171
8. Fava RA, Olsen NJ, Postlethwaite AE, Broadley KN, Davidson JM, Nanney LB, Lucas C, Townes AS: Transforming growth factor β1(TGF-β1)induced neutrophil recruitment to synovial tissue: implications for TGF-β-driven synovial inflammation and hyperplasia. J Exp Med 1991, 173:1121–1132
9. Kuruvilla AP, Shah R, Hochwald GM, Liggitt HD, Palladino MA, Thorbecke GJ: Protective effect of transforming growth factor β1 on experimental autoimmune diseases in mice. Proc Natl Acad Sci USA 1991, 88: 2918–2921
10. Johns LD, Flanders KC, Ranges CE, Sriram S: Successful treatment of experimental allergic encephalomyelitis with transforming growth factor-β1. J Immunol 1991, 147:1792–1796
11. Sellheyer K, Bickenbach JR, Rothanagel JA, Bundman D, Longley MA, Krieg T, Roche NS, Roberts AB, Roop DR: Inhibition of skin development by overexpression of transforming growth factor β1 in the epidermis of transgenic mice. Proc Natl Acad Sci USA 1993, 90:5237–5241
12. Jhappan C, Geiser AG, Kordon EC, Bagheri D, Henninghausen L, Robert AB, Smith GH, Merlino G: Targeting expression of a transforming growth factor β1 transgene to the pregnant mammary gland inhibits alveolar development and lactation. EMBO J 1993, 12: 1835–1845
13. Brunner AM, Marquardt H, Malacko AR, Lioubin MN, Purchio AF: Site-directed mutagenesis of cysteine residues in the pro region of the transforming growth factor β1 precursor. J Biol Chem 1989, 23:13660–13664
14. Samuel SK, Hurta RAR, Kondaiah P, Khalil N, Turley EA, Wright JA, Greenberg AH: Autocrine induction of tumor protease production and invasion by a metallothionein-regulated TGF-β1 (Ser223,225). EMBO J 1992, 11:1599–1605
15. Feng L, Xia Y, Kreisberg I, Wilson CB: Interleukin-1α stimulates KC synthesis in rat mesengial cells: glucocorticoid inhibits KC induction by IL-1. Am J Physiol 1994, 266:F713–F722
16. Xia Y, Feng L, Yoshimura T, Wilson CB: LPS-induced MCP-1 IL-1β and TNF-α mRNA expression in isolated erythrocyte-perfused rat kidney. Am J Physiol 1993, 264:F774–F780
17. Brunstedt J, Nielsen JH, Lernmark A, A.T.H.S. Group: Isolation of islets from mice and rats. Methods in Diabetes Research: Laboratory Methods. Edited by H Larner and SL Pohl. New York, John Wiley & Sons, 1984, pp 245–258
18. Abe M, Harpel JG, Metz CN, Nunes I, Loskutoff DJ, Rifkin DB: An assay for transforming growth factor β using cells transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct. Anal Biochem 1994, 216:276–284
19. Keeton M, Curriden SA, van Zonnenveld AJ, Loskutoff DJ: Identification of regulatory sequences in the type 1 plasminogen activator inhibitor gene responsive to transforming growth factor β. J Biol Chem 1991, 266: 23048–23052
20. Pipeleers DG: Heterogeneity in pancreatic β-cell population. Diabetes 1992, 41:777–781
21. Heino J, Massague J: Transforming growth factor-β switches the pattern of integrins expressed in MG-63 human osteosarcoma cells and causes a selective loss of cell adhesion to laminin. J Biol Chem 1989, 264:21806–21811
22. Laiho M, Saksela O, Andreasen PA, Keski-Oja J: Enhanced production and extracellular deposition of the endothelial-type plasminogen activator inhibitor in cultured human lung fibroblasts by transforming growth factor-β. J Cell Biol 1986, 103:2403–2410

23. Lund LR, Riccio A, Andreasen PA, Neilsen LS, Kresten-sen P, Laiho M, Blasi F, Dano K: Transforming growth factor-β is a strong and fast acting positive regulator of the level of type-1 plasminogen activator inhibitor mRNA in WI-38 human lung fibroblasts. EMBO J 1987, 6:1281–1286
24. Montesano R, Pepper MS, Vassalli J-D, Orci L: Phorbol ester induces cultured endothelial cells to invade a fibrin matrix in the presence of fibrinolytic inhibitors. J Cell Physiol 1987, 132:509–516
25. Moscatelli D, Rifkin DB: Membrane and matrix localization of protease: a common theme in tumor invasion and angiogenesis. Biochim Biophys Acta 1988, 948:67–85
26. Ignotz RA, Massague J: Cell adhesion protein receptors as targets for transforming growth factor-β action. Cell 1987, 51:189–197
27. Dean DC, Newby RF, Bourgeois S: Regulation of fibronectin biosynthesis by dexamethasone, transforming growth factor β, and cAMP in human cell lines. J Cell Biol 1988, 106:2159–2170
28. Huang S, Chakrabaraty S: Regulation of fibronectin and laminin receptor expression, fibronectin and laminin secretion in human colon cancer cells by transforming growth factor-β1. Int J Cancer 1994, 57:742–746
29. Pierce DF, Johnson MD, Matsui Y, Robinson SD, Gold LI, Purchio AF, Daniel CW, Hogan BLM, Moses HL: Inhibition of mammary duct development but not alveolar outgrowth during pregnancy in transgenic mice expressing active TGF-β1. Genes & Dev 1993, 7:2308–2317
30. Sandgren EP, Luetteke NC, Palmiter RD, Brinster RL, Lee DC: Overexpression of TGFα in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. Cell 1990, 61: 1121–1135
31. Jhappan C, Stahle C, Harkins RN, Fausto N, Smith GH, Merlino GT: TGFα overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. Cell 1990, 61:1137–1146
32. Alitalo K, Vaheri A: Pericellular matrix in malignant transformation. Adv Cancer Res 1982, 37:111–158
33. Parekh T, Saxena B, Reibman J, Cronstein BN, Gold LI: Neutrophil chemotaxis in response to TGF-β isoforms (TGF-β1, TGF-β2, TGF-β3) is mediated by fibronectin. J Immunol 1994, 152:2456–2466
34. Bauer GE: Islets of Langerhans. Histology: Cell and Tissue Biology. Edited by L. Weiss. New York, Elsevier Science Publishing Co., 1983, pp 781–782
35. Wahl SM, Allen JB, Weeks BS, Wong HL, Klotman PE: Transforming growth factor β enhances integrin expression and type IV collagenase secretion in human monocytes. Proc Natl Acad Sci USA 1993, 90:4577–4581
36. Koff A, Ohtsuki M, Polyak K, Roberts JM, Massague J: Negative regulation of G1 in mammalian cells: inhibition of cyclin E-dependent kinase by TGF-β. Science 1993, 260:536–539
37. Border WA, Ruoslahti E: Transforming growth factor-β in disease. The dark side of tissue repair. J Clin Invest 1992, 90:1–7
38. Rosai J: Ackerman's surgical pathology. St. Louis, The C.V. Mosby Company, 1989, pp 760
39. Cotran RS, Kumar V, Robbins SL: Robbins Pathologic Basis of Disease. Philadelphia, W. B. Saunders Company, 1989, pp 987–988