(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 February 2002 (14.02.2002)

PCT

(10) International Publication Number
WO 02/12336 A2

(51) International Patent Classification[7]: C07K 14/495

(21) International Application Number: PCT/US01/04192

(22) International Filing Date: 9 February 2001 (09.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/635,368 9 August 2000 (09.08.2000) US

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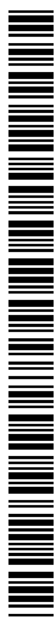
(81) **Designated States** *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



(54) **Title:** TGF-β THERAPEUTICS, COMPOSITIONS AND METHODS OF USE

Human TGFβ-1 Amino Acid Sequence (SEQ. ID. No.1)

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  1 mppsglrlll lllpllwllv ltpgrpaagl stcktidmel vkrkrieair gqilsklrla
 61 sppsqgevpp gplpeavlal ynstrdrvag esaepepepe adyyakevtr vlmvethnei
121 ydkfkqsths iymffntsel reavpepvll sraelrllrl klkveqhvel yqkysnnswr
181 ylsnrllaps dspewlsfdv tgvvrqwlsr ggeiegfrls ahcscdsrdn tlqvdingft
241 tgrrgdlati hgmnrpflll matpleraqh lqssrhrral dtnycfsste knccvrqlyi
301 dfrkdlgwkw ihepkgyhan fclgpcpyiw sldtqyskvl alynqhnpga saapccvpqa
361 leplpivyyv grkpkveqls nmivrsckcs
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(57) Abstract: The present invention relates to TGFβ therapeutics, TGFβ proteins or a combination of TGFβ proteins which are capable of stimulating growth and/or differentiation of pancreatic tissue. The invention also relates to pharmaceutical compositions comprising such proteins and/or therapeutics, methods for using such proteins and/or therapeutics in culturing pancreatic tissue and cells and methods for administering such proteins and/or therapeutics to a subject.

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TGF-β Therapeutics, Compositions and Methods of Use

Related Applications

5 This Application is a continuation-in part of PCT Application US 00/03424 filed February 10, 2000 and U.S. Application 09/500822 filed February 10, 2000, the specifications of both of which are incorporated herein by reference.

Background of the Invention

10 During the early stages of embryogenesis cells are totipotent and are capable of multidirectional differentiation. As development proceeds, the totipotent cells become determined and committed to differentiate into a given specialized cell type. Final differentiation is associated with the acquisition of specialized cell functions. Thus, the differentiated somatic cells maintain their specialized features throughout the life span of 15 the organism, probably through sustained interactions between the genome and its microenvironment and cell-cell interactions (DiBerardino et al., 1984, Science 224:946-952; Wetts and Fraser, 1988, Science 239:1142-1144; Fisher, 1984, PNAS 81:4414-4418).

Because of the tremendous potential of progenitor cells to differentiate into distinct lineages, there has always existed a need for a continuous source of these isolated 20 pluripotent progenitor cells. The pluripotent progenitor cells could be extremely useful in the treatment of different disorders that are characterized by insufficient or abnormal functioning of the fully differentiated cells in a given organ, as for example in the human pancreas or liver.

The need for progenitor cells which can form functioning, glucose-responsive 25 pancreatic cells is particularly important. Insulin-dependent diabetes mellitus (IDDM) is a disease characterized by elevated blood glucose and the absence of the hormone insulin. The cause of the raised sugar levels is insufficient secretion of the hormone insulin by the pancreas. In the absence of this hormone, the body's cells are not able to absorb sugar from the blood stream in normal fashion, and the excess sugar accumulates in the blood. 30 Chronically elevated blood glucose damages tissues and organs. IDDM is treated with insulin injections. The size and timing of insulin injections are influenced by measurements of blood sugar.

There are over 400 million diabetics in the world today. For instance, diabetes is one of the most prevalent chronic diseases in the United States, and a leading cause of death. Estimates based on the 1993 National Health Interview Survey (NHIS) indicate that diabetes has been diagnosed in 1% of the U.S. population age <45 years, 6.2% of those age 45-64 years, and 10.4% of those age >65 years. In other terms, in 1993 an estimated 7.8 million persons in the United States were reported to have this chronic condition. In addition, based on the annual incidence rates for diabetes, it is estimated that about 625,000 new cases of diabetes are diagnosed each year, including 595,000 cases of non-insulin-dependent diabetes mellitus (NIDDM) and 30,000 cases of insulin-dependent diabetes mellitus (IDDM).

The total cost of diabetes in the United States has been estimated at $92 billion annually, including expenditures on medical products, hospitalization and the value of lost work. Substantial costs to both society and its citizens are incurred not only for direct costs of medical care for diabetes, but also for indirect costs, including lost productivity resulting from diabetes-related morbidity and premature mortality. Persons with diabetes are at risk for major complications, including diabetic ketoacidosis, end-stage renal disease, diabetic retinopathy and amputation. There are also a host of less directly related conditions, such as hypertension, heart disease, peripheral vascular disease and infections, for which persons with diabetes are at substantially increased risk.

While medications such as injectable insulin and oral hypoglycemics allow diabetics to live longer, diabetes remains the third major killer, after heart disease and cancer. Diabetes is also a very disabling disease, because medications do not control blood sugar levels well enough to prevent swinging between high and low blood sugar levels, with resulting damage to the kidneys, eyes, and blood vessels.

Studies have documented that medical costs for persons with diabetes are higher because they visit physician's offices, hospital outpatient departments, and emergency rooms more frequently than their nondiabetic counterparts, and are more likely to be admitted to the hospital. Americans with diabetes have two to five times higher per capita total medical expenditures and per capita out-of-pocket expenses than people without diabetes. These expenses and their associated loss of productivity have impact not only on diabetic patients and their families, but on federal and state governments and society as a whole.

Data from the Diabetes Control and Complications Trial (DCCT) show that intensive control of blood glucose significantly delays complications of diabetes, such as retinopathy, nephropathy, and neuropathy, compared with conventional therapy consisting of one or two insulin injections per day. Intensive therapy in the DCCT included multiple
5 injection of insulin three or more times per day or continuous subcutaneous insulin infusion (CSII) by external pump. Insulin pumps are one of a variety of alternative approaches to subcutaneous multiple daily injections (MDI) for approximating physiological replacement of insulin.

Although it is possible to transplant the human pancreas, the shortage of donors and
10 problems of immune rejection limit this procedure to selected patients. β-cell transplantation has been accomplished successfully in humans, but the large number of β-cells required has been an obstacle.

Therefore, there is a need for methods of stimulating growth of pancreatic tissue in vitro and in vivo.

15

Summary of the Invention

The present invention relates to the stimulation of growth and/or differentiation of various cell types, including, for example, pancreatic cells, by treating said cells with TGFβ therapeutics and pharmaceutical compositions comprising such TGFβ therapeutics. The
20 invention also relates to methods for using such proteins, pharmaceutical compositions and therapeutics in culturing cells and tissues such as the pancreatic tissue and cells, and methods for administering such agents to a subject.

In one aspect, TGFβ therapeutics and pharmaceutical compositions comprising such TGFβ therapeutics, include a TGFβ protein. Broadly, TGFβ proteins of the invention
25 comprise proteins of the TGFβ superfamily. Preferably, the protein comprises a polypeptide sequence that is at least 60%, 70%, 80%, 90%, 95%, or 97-99%, identical or homologous to a protein of the TGFβ sub-family. More preferably, the TGFβ therapeutics include within their scope polypeptides that are at least 60%, 70%, 80%, 90%, 95%, or 97-99%, identical or homologous to a members of the TGFβ subfamily, i.e, polypeptides that
30 are at least at least 60%, 70%, 80%, 90%, 95%, or 97-99%, identical or homologous to TGFβ-1, -2, -3, -4 and -5, represented by SEQ ID Nos 1-5, respectively. In one embodiment, the TFGβ therapeutic comprises a polypeptide as set forth in SEQ ID Nos: 1-5. In particularly preferred embodiments, the polypeptide is at least 60%, 70%, 80%, 90%,

95%, or 97-99%, identical or homologous to a identical or homologous to TGFβ-2, -3 or -5 represented by SEQ. ID. Nos. 2, 3, or 5 respectively). In one embodiment, the TFGβ therapeutic comprises a polypeptide as set forth in SEQ ID Nos: 2, 3, or 5. More preferably, the polypeptide is at least 60%, 70%, 80%, 90%, 95%, or 97-99%, identical or
5 homologous to a polypeptide represented by SEQ ID Nos: 7, 8, and 10. In one embodiment, the TFGβ therapeutic comprises a polypeptide as set forth in SEQ ID Nos: 7, 8, and/or 10. Most preferably the polypeptide includes a polypeptide at least 60%, 70%, 80%, 90%, 95%, or 97-99%, identical or homologous to a polypeptide as set forth in SEQ ID Nos: 10-13. In one embodiment, the TFGβ therapeutic comprises a polypeptide as set
10 forth in SEQ ID Nos: 10-13.

The TGFβ polypeptides as used herein can comprise a full-length protein, such as represented in SEQ. ID. Nos. 1-10, or may comprise a fragment corresponding to particular motifs/domains. Accordingly, the TGFβ polypeptides comprise at least 25, 50, 100, 150 or 200 amino acids of SEQ ID Nos: 1-13. In preferred embodiments, the polypeptide, or
15 fragment thereof, specifically modulates, by acting as either an agonist or antagonist, the signal transduction activity of a receptor for a TGFβ protein. In particularly preferred embodiments, the TGFβ polypeptide comprises a polypeptide sequence corresponding to the mature, processed portion of a TGFβ subfamily member, shown in SEQ. ID. Nos. 6-10. Particularly preferred mature polypeptide sequences are those derived from TGFβ-2, -3 or -
20 5 (SEQ. ID. Nos. 7, 8 or 10). A TGFβ subfamily polypeptide may also have an amino acid sequence greater than 76% homologous to a polypeptide represented by any of SEQ ID Nos: 6-10, though polypeptides with higher sequence homologies of, for example, 80, 85, 90, 95, 98 and 99% or are also contemplated.

In one aspect, the invention pertains to isolated nucleic acids comprising nucleotide
25 sequences encoding TGFβ polypeptides, and/or equivalents of such nucleic acids. The term nucleic acid as used herein is intended to include fragments as equivalents. The term equivalent is understood to include nucleotide sequences encoding functionally equivalent TGFβ polypeptides or functionally equivalent peptides having an activity of a TGFβ protein such as described herein. Equivalent nucleotide sequences will include sequences that
30 differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants; and will, therefore, include sequences that differ from the nucleotide sequence encoding the TGFβ cDNA sequences due to the degeneracy of the genetic code.

Equivalents will also include nucleotide sequences that hybridize under stringent conditions (i.e., equivalent to about 20-27°C below the melting temperature (T_m) of the DNA duplex formed in about 1M salt) to the nucleotide sequences encoding the polypeptides set forth in SEQ ID Nos: 1-10. In one embodiment, equivalents will further include nucleic acid
5 sequences derived from and evolutionarily related to, a nucleotide sequences encoding polypeptides set forth in SEQ ID Nos: 1-10.

Accordingly, another aspect of the invention provides a nucleic acid which hybridizes under high or low stringency conditions to the nucleic acid represented by Genbank Accession Nos. 339547, 339549, 4507464, 2289340, 2289341, 2289342,
10 2289343, 2289344. Appropriate stringency conditions which promote DNA hybridization, for example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of
15 about 2.0 x SSC at 50°C to a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C.

Nucleic acids having a sequence that differs from the nucleotide sequences shown in GenBank Accession Nos. 339547, 339549, 4507464, 2289340, 2289341, 2289342,
20 2289343, 2289344 due to degeneracy in the genetic code are also within the scope of the invention. Such nucleic acids encode functionally equivalent peptides (i.e., a peptide having a biological activity of a TGFβ polypeptide) but differ in sequence from the sequence shown in the sequence listing due to degeneracy in the genetic code. For example, a number of amino acids are designated by more than one triplet. Codons that
25 specify the same amino acid, or synonyms (for example, CAU and CAC each encode histidine) may result in "silent" mutations which do not affect the amino acid sequence of a TGFβ polypeptide. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject TGFβ polypeptides will exist among vertebrates. One skilled in the art will appreciate that these variations in one or
30 more nucleotides (up to about 3-5% of the nucleotides) of the nucleic acids encoding polypeptides having an activity of a TGFβ polypeptide may exist among individuals of a given species due to natural allelic variation.

In another embodiment, the TGFβ polypeptide of the methods and compositions comprises a TGFβ–5 motif represented in any of the formulas shown in SEQ ID Nos 11-13.

In another preferred embodiment, a TGFβ protein comprises a purified or recombinant polypeptide fragment of a TGFβ protein, which polypeptide has the ability to modulate, e.g., mimic or antagonize, a the activity of a wild-type TGFβ protein. Preferably, the polypeptide fragment comprises a TGFβ–5 motif. Moreover, as described below, the preferred TGFβ polypeptide can be an agonist (e.g. mimics) of a biological activity of a naturally occurring form of the protein, e.g., the polypeptide is able to modulate differentiation and/or growth and/or survival of a cell responsive to authentic TGFβ–5 proteins. Homologs of the subject TGFβ–5 proteins include versions of the protein which are resistant to post-translation modification, as for example, due to mutations which alter modification sites (such as tyrosine, threonine, serine or aspargine residues), or which inactivate an enzymatic activity associated with the protein.

The subject proteins can also be provided as chimeric molecules, such as in the form of fusion proteins. For instance, the TGFβ protein can be provided as a recombinant fusion protein which includes a second polypeptide portion, e.g., a second polypeptide having an amino acid sequence unrelated (heterologous) to the TGFβ polypeptide, e.g. the second polypeptide portion is glutathione-S-transferase, e.g. the second polypeptide portion is an enzymatic activity such as alkaline phosphatase, e.g. the second polypeptide portion is an epitope tag.

In one aspect, the invention comprises contacting cells such as the pancreatic duct cells with a TGFβ therapeutic, a composition comprising a TGFβ protein or a cell that produces a TGFβ protein, under conditions sufficient to induce outgrowth tissue such as an outgrowth of the pancreatic duct tissue thereby forming pancreatic and/or endocrine tissue.

Yet another aspect of the present invention concerns a method for modulating one or more of growth, differentiation, or survival of cells such as pancreatic cells responsive to TGFβ induction. In general, whether carried out *in vivo*, *in vitro*, or *in situ*, the method comprises treating the cell with an effective amount of a TGFβ therapeutic so as to alter, relative to the cell in the absence of TGFβ treatment, at least one of (i) rate of growth, (ii) differentiation, or (iii) survival of the cell. Accordingly, the method can be carried out with polypeptides that mimic the effects of a naturally-occurring TGFβ protein on the cell, eg. induce growth or differentiation of pancreatic cells. In preferred embodiments, the TGFβ

polypeptides provided in the subject method are derived from verterbrate sources, e.g., are vertebrate TGFβ polypeptides. For instance, preferred polypeptides includes an amino acid sequence identical or homologous to an amino acid sequence (e.g., including bioactive fragments) designated in one of SEQ ID Nos 1-13. Furthermore, the present invention contemplates the use of other metazoan (e.g., invertebrate) homologs of the TGFβ polypeptides or bioactive fragments thereof equivalent to the subject vertebrate fragments.

The present method is applicable, for example, to cells such as pancreatic cell cultures technique, such as in the culturing of pancreatic duct cells whose growth or differentiation can be modulated by TGFβ function. In an exemplary embodiment, the method is practiced for modulating, in an animal, cell growth, cell differentiation or cell survival, and comprises administering a therapeutically effective amount of a TGFβ therapeutic or pharmaceutical preparation to alter, relative to the absence of TGFβ treatment, at least one of (i) rate of growth, (ii) differentiation, or (iii) survival of one or more pancreatic cell-types in the animal.

In another aspect, the invention features a method for treating a disorder characterized by insufficient insulin activity in a subject, comprising introducing into the subject a pharmaceutical composition comprising a TGFβ protein, a preparation of TGFβ protein-producing cells, or differentiated cells which have been stimulated using TGFβ protein or TGFβ protein-producing cells, and a pharmaceutically acceptable carrier. In a preferred embodiment the disorder is an insulin-dependent diabetes, e.g., type I diabetes.

Application of the invention can be used to generate new tissue, such as pancreatic tissue for ex vivo implantation, for example from a sample of the patient's own pancreatic tissue. In this method, pancreatic tissue removed from the patient is contacted with TGFβ therapeutic, a composition containing TGFβ protein or with cells which secrete TGFβ protein under conditions sufficient to induce outgrowth of the pancreatic tissue thereby forming new pancreatic tissue. The new tissue then can be transplanted back into the patient. In one embodiment, the pancreatic tissue can be activated prior to implantation, for example by application of PYY peptide or an analog or agonist thereof which induces glucose sensitivity in pancreatic tissue. Methods of ex vivo therapy per se are well-established in the art and accordingly are not described in detain herein.

In a preferred embodiment the subject is a mammal, e.g., a primate, e.g., a human.

In certain embodiments, pharmaceutical preparations of the invention comprise a TGFβ protein and a pharmaceutically acceptable carrier. The TGFβ protein preferably

comprises a protein at least 76% homologous to SEQ. ID. Nos. 5 or 10. In particularly preferred embodiments, pharmaceutical preparations comprise a TGFβ-5 motif as shown in SEQ. ID. Nos. 11-13.

The practice of the present invention will employ, unless otherwise indicated,
5 conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are described in the literature. See, for example, Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); DNA Cloning, Volumes I and II (D. N. Glover ed., 1985);
10 Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Patent No: 4,683,195; Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In
15 Enzymology (Academic Press, Inc., N.Y.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu et al. eds.), Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986);
20 Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

25

Brief Description of the Drawings

Figure 1 is the amino acid sequence of the human TGFβ-1 proprotein (Accession # GI 135674; SEQ. ID. No.1).

30 Figure 2 is the amino acid sequence of the human TGFβ-2 proprotein (Accession # GI 557563; SEQ. ID. No. 2).

Figure 3 is the amino acid sequence of the human TGFβ-3 proprotein (Accession # GI 135684; SEQ. ID. No. 3).

Figure 4 is the amino acid sequence of the human TGFβ-4 proprotein (Accession # GI 135674; SEQ. ID. No. 4).

Figure 5 is the amino acid sequence of the Xenopus laevis TGFβ-5 proprotein (Accession # GI 135674; SEQ. ID. No. 5).

5 Figure 6 is the amino acid sequence of the mature human TGFβ-1 protein (SEQ. ID. No. 6).

Figure 7 is the amino acid sequence of the mature human TGFβ-2 protein (SEQ. ID. No. 7).

Figure 8 is the amino acid sequence of the mature human TGFβ-3 protein (SEQ. ID. 10 No. 8).

Figure 9 is the amino acid sequence of the mature human TGFβ-4 protein (SEQ. ID. No. 9).

Figure 10 is the amino acid sequence of the mature X. laevis TGFβ-5 protein (SEQ. ID. No. 10).

15 Figure 11 shows TGFβ-5 motifs (SEQ. ID. Nos. 11-13).

Figure 12 shows an alignment of human TGFβ -1, -2 and -3 proteins with TGFβ-5 from *X. laevis*.

Figure 13 is a graph showing growth stimulation of rat pancreatic ductal explants treated with TGFβ proteins.

20 Figure 14 is a graph showing growth stimulation of mouse pancreatic ductal explants treated with TGFβ proteins.

Figure 15 is a graph showing expansion of PDX-1/LacZ expressing cells in mouse pancreatic ductal explants treated with TGFβ proteins.

Figure 16 shows staining for LacZ expression in PDX-1/LacZ ductal explants 25 treated with TGFβ proteins.

Figure 17 is a graph showing body weights of TGFβ treated mice.

Figure 20 is a graph showing blood glucose concentrations in fasting TGFβ treated mice.

Figure 21 is a graph showing plasma insulin concentrations in fasting TGFβ treated 30 mice.

Figure 22 shows proliferation in the common pancreatic duct was determined by 5-bromo-2'-deoxyuridine (BrdU) incorporation in TGFβ treated cells. Insulin staining is also shown.

Figure 23 shows the pancreatic islet area in the TGFβ3 and TGFβ5 treated mice.
5 Pancreatic sections from vehicle and TGFβ treated mice sacrificed on day 5 were co-stained for insulin and glucagon.

Figure 24 Quantitation of islet area in TGFβ5 treated mice.

Detailed Description of the Invention

10

Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

As used herein the term "animal" refers to mammals, preferably mammals such as
15 humans. Likewise, a "patient" or "subject" to be treated by the method of the invention can mean either a human or non-human animal.

As used herein, the term "cellular composition" refers to a preparation of cells, which preparation may include, in addition to the cells, non-cellular components such as cell culture media, e.g. proteins, amino acids, nucleic acids, nucleotides, co-enzyme, anti-
20 oxidants, metals and the like. Furthermore, the cellular composition can have components which do not affect the growth or viability of the cellular component, but which are used to provide the cells in a particular format, e.g., as polymeric matrix for encapsulation or a pharmaceutical preparation. A cellular composition may also include a tissue sample such as a pancreatic duct explant.

25 A "chimeric protein" or "fusion protein" is a fusion of a first amino acid sequence encoding one of the subject TGFβ polypeptides with a second amino acid sequence defining a domain (e.g. polypeptide portion) foreign to and not substantially homologous with any domain of one of the TGFβ proteins. A chimeric protein may present a foreign domain which is found (albeit in a different protein) in an organism which also expresses
30 the first protein, or it may be an "interspecies", "intergenic", etc. fusion of protein structures expressed by different kinds of organisms. In general, a fusion protein can be represented by the general formula X- TGFβ protein-Y, wherein TGFβ protein represents a portion of

the protein which is derived from one of the vertebrate TGFβ protein proteins, and X and Y are independently absent or represent amino acid sequences which are not related to one of the TGFβ protein sequences in an organism, including naturally occurring mutants.

The term "culture medium" is recognized in the art, and refers generally to any substance or preparation used for the cultivation of living cells. Accordingly, a "tissue culture" refers to the maintenance or growth of tissue, e.g., explants of organ primordia or of an adult organ in vitro so as to preserve its architecture and function. A "cell culture" refers to a growth of cells in vitro; although the cells proliferate they do not organize into tissue per se.

Tissue and cell culture preparations of micro-organ explants and amplified progenitor cell populations used herein can take on a variety of formats. For instance, a "suspension culture" refers to a culture in which cells multiply while suspended in a suitable medium. Likewise, a "continuous flow culture" refers to the cultivation of cells or ductal explants in a continuous flow of fresh medium to maintain cell growth, e.g. viability.

The terms "explant" and "micro-organ explant" refer to a portion of an organ taken from the body and grown in an artificial medium.

"Homology" refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An "unrelated" or "non-homologous" sequence shares less than 40 percent identity, though preferably less than 25 percent identity, with one of the vertebrate TGFβ sequences of the present invention.

The term "lineage committed cell" refers to a progenitor cell that is no longer pluripotent but has been induce to differentiate into a specific cell type, e.g., a pancreatic, hepatic or intestinal cell.

The term "organ" refers to two or more adjacent layers of tissue, which layers of tissue maintain some form of cell-cell and/or cell-matrix interaction to form a microarchitecture.

The term "pancreas" is art recognized, and refers generally to a large, elongated, racemose gland situated transversely behind the stomach, between the spleen and

duodenum. The pancreatic exocrine function, e.g., external secretion, provides a source of digestive enzymes. Indeed, "pancreatin" refers to a substance from the pancreas containing enzymes, principally amylase, protease, and lipase, which substance is used as a digestive aid. The exocrine portion is composed of several serous cells surrounding a lumen. These
5 cells synthesize and secrete digestive enzymes such as trypsinogen, chymotrypsinogen, carboxypeptidase, ribonuclease, deoxyribonuclease, triacylglycerol lipase, phospholipase A_2, elastase, and amylase.

The endocrine portion of the pancreas is composed of the islets of Langerhans. The islets of Langerhans appear as rounded clusters of cells embedded within the exocrine
10 pancreas. Four different types of cells- α, β, δ, and ϕ-have been identified in the islets. The α cells constitute about 20% of the cells found in pancreatic islets and produce the hormone glucagon. Glucagon acts on several tissues to make energy available in the intervals between feeding. In the liver, glucagon causes breakdown of glycogen and promotes gluconeogenesis from amino acid precursors. The δ cells produce somatostatin which acts
15 in the pancreas to inhibit glucagon release and to decrease pancreatic exocrine secretion. The hormone pancreatic polypeptide is produced in the ϕ cells. This hormone inhibits pancreatic exocrine secretion of bicarbonate and enzymes, causes relaxation of the gallbladder, and decreases bile secretion. The most abundant cell in the islets, constituting 60-80% of the cells, is the β cell, which produces insulin. Insulin is known to cause the
20 storage of excess nutrients arising during and shortly after feeding. The major target organs for insulin are the liver, muscle, and fat-organs specialized for storage of energy.

The term "pancreatic progenitor cell" refers to a cell which can differentiate into a cell of pancreatic lineage, e.g. a cell which can produce a hormone or enzyme normally produced by a pancreatic cell. For instance, a pancreatic progenitor cell may be caused to
25 differentiate, at least partially, into α, β, δ, or ϕ islet cell, or a cell of exocrine fate. The pancreatic progenitor cells of the invention can also be cultured prior to administration to a subject under conditions which promote cell proliferation and differentiation. These conditions include culturing the cells to allow proliferation and confluence in vitro at which time the cells can be made to form pseudo islet-like aggregates or clusters and secrete
30 insulin, glucagon, and somatostatin.

The present invention comprises a factor or factors which stimulate outgrowth of pancreatic duct tissue. These outgrowths were not simply spreading of existing cells (i.e.,

characterized by BrdU staining), the new material is pancreatic/endocrine in nature (i.e., characterized by PDX-1 staining).

The term "progenitor cell" refers to an undifferentiated cell which is capable of proliferation and giving rise to more progenitor cells having the ability to generate a large number of mother cells that can in turn give rise to differentiated, or differentiable daughter cells. As used herein, the term "progenitor cell" is also intended to encompass a cell which is sometimes referred to in the art as a "stem cell". In a preferred embodiment, the term "progenitor cell" refers to a generalized mother cell whose descendants (progeny) specialize, often in different directions, by differentiation, e.g., by acquiring completely individual characters, as occurs in progressive diversification of embryonic cells and tissues.

The term "recombinant protein" refers to a polypeptide of the present invention which is produced by recombinant DNA techniques, wherein generally, DNA encoding a vertebrate TGFβ polypeptide is inserted into a suitable expression vector which is in turn used to transform a host cell to produce the heterologous protein. Moreover, the phrase "derived from", with respect to a recombinant TGFβ gene, is meant to include within the meaning of "recombinant protein" those proteins having an amino acid sequence of a native TGFβ protein, or an amino acid sequence similar thereto which is generated by mutations including substitutions and deletions (including truncation) of a naturally occurring form of the protein.

As used herein the term "substantially pure", with respect to collections of cells, refers to a population of cells that is at least about 75%, preferably at least about 85%, more preferably at least about 90%, and most preferably at least about 95% pure, with respect to a type of cell making up a total cell population. Recast, the term "substantially pure" refers to a population of cells of the present invention that contain fewer than about 20%, more preferably fewer than about 10%, most preferably fewer than about 5%, of lineage committed cells in the original unamplified and isolated population prior to subsequent culturing.

The term "tissue" refers to a group or layer of similarly specialized cells which together perform certain special functions.

As used herein, the term "transfection" means the introduction of a nucleic acid, e.g., an expression vector, into a recipient cell by nucleic acid-mediated gene transfer. "Transformation", as used herein, refers to a process in which a cell's genotype is changed

as a result of the cellular uptake of exogenous DNA or RNA, and, for example, the transformed cell expresses a recombinant form of a vertebrate TGFβ polypeptide or, where anti-sense expression occurs from the transferred gene, the expression of a naturally-occurring form of the TGFβ protein is disrupted.

5 As used herein, the terms "transforming growth factor-beta protein" and "TGFβ protein" (or “polypeptide”) denote a family of structurally related paracrine polypeptides found ubiquitously in vertebrates, and prototypic of a large family of metazoan growth, differentiation, and morphogenesis factors (see, for review, Massaque et al. (1990) *Ann Rev Cell Biol* 6:597-641; Massaque et al. (1994) *Trends Cell Biol.* 4:172-178; Kingsley (1994) 10 *Gene Dev.* 8:133-146; and Sporn et al. (1992) *J Cell Biol* 119:1017-1021). As described in Kingsley, *supra*, the TGFβ superfamily has at least 25 members, and can be grouped into distinct sub-families with highly related sequences. The most obvious sub-families include the following: the TGFβ sub-family, which comprises at least five members (TGFβ-1, -2, -3, -4, -5) that are much more similar to TGFβ-1 than to other members of the TGFβ 15 superfamily; the activin sub-family, comprising homo- or hetero-dimers or two sub-units, inhibinβ-A and inhibinβ-B. The decapentaplegic sub-family, which includes the mammalian factors BMP2 and BMP4, which can induce the formation of ectopic bone and cartilage when implanted under the skin or into muscles. The 60A sub-family, which includes a number of mammalian homologs, with osteoinductive activity, including BMP5-20 8. Other members of the TGFβ superfamily include the gross differentiation factor 1 (GDF-1), GDF-3/VGR-2, dorsalin, nodal, mullerian-inhibiting substance (MIS), and glial-derived neurotrophic growth factor (GDNF). It is noted that the DPP and 60A sub-families are related more closely to one another than to other members of the TGFβ superfamily, and have often been grouped together as part of a larger collection of molecules called DVR 25 (dpp and vg1 related). Unless evidenced from the context in which it is used, the term TGFβ as used throughout this specification will be understood to generally refer to members of the TGFβ superfamily as appropriate. Reference to members of the TGFβ sub-family will be explicit, or evidenced from the context in which the term TGFβ is used. The term "TGFβ protein" is also understood to encompass other molecules that are related in 30 sequence homology or are bioactive fragments of a TGFβ superfamily member.

As used herein, a "TGFβ therapeutic" is any compound or mixture of compounds that can stimulate the growth and or differentiation of pancreatic cells in a

manner similar to TGFβ -2, -3 or -5. Exemplary "TGFβ therapeutics" encompass TGFβ proteins, including but not limited to polypeptides that are at least 60%, 70% 80%, 90%, 95%, or 97-99% identical to polypeptides represented by SEQ ID Nos: 1-5 and fragments thereof; more preferably these therapeutics include polypeptides that are at least 60%, 70% 80%, 90%, 95%, or 97-99% identical to polypeptides represented by SEQ ID Nos: 7, 8, 10, and fragments thereof; and most preferably they include polypeptides that are at least 60%, 70% 80%, 90%, 95%, or 97-99% identical to polypeptides represented by SEQ ID Nos: 10-13 and fragments thereof that have the appropriate activity. As one of ordinary skill would readily appreciate the term TGFβ therapeutics includes within its scope agonists, small molecules, etc. that can stimulate the growth and or differentiation of various cell types such as pancreatic cells.

Overview

In certain aspects, the present invention comprises contacting cells such as the pancreatic cells *in vivo* or *in vitro* with a TGFβ therapeutic or protein or cells expressing TGFβ protein as described below.

The TGFβ superfamily is an important class of molecules involved in cell-cell signaling and development in a wide range of organisms and cell types. Members of the family are initially synthesized as larger precursor molecules with an amino-terminal signal sequence and a pro-domain of varying size (Kingsley, D.M. (1994) *Genes Dev.* 8:133-146). The precursor is then cleaved to release a mature carboxy-terminal segment of 110-140 amino acids. The active signaling moiety is comprised of hetero- or homodimers of the carboxy-terminal segment (Massague, J. (1990) *Annu. Rev. Cell Biol.* 6:597-641). The active form of the molecule then interacts with its receptor, which for this family of molecules is composed of two distantly related transmembrane serine/threonine kinases called type I and type II receptors (Massague, J. et al. (1992) *Cell* 69:1067-1070; Miyazono, K. A. et al. *EMBO J.* 10:1091-1101). TGFβ binds directly to the type II receptor, which then recruits the type I receptor and modifies it by phosphorylation. The type I receptor then transduces the signal to downstream components, which are as yet unidentified (Wrana et al, (1994) *Nature* 370:341-347).

In general, members of the TGFβ superfamily have a set of nine highly conserved cysteine residues that are involved in disulfide bonding both within and between monomers of the mature, dimerized signaling protein (Griffith et al. (1996) *PNAS* 93: 878-883; Luo et al. (1995) *PNAS* 92: 11761-11765; Schlunegger et al. (1993) *J. Mol. Biol.* 231: 445-58; Daopin et al. (1993) *Proteins* 17: 176-92; Murray-Rust et al. (1993) *Structure* 15: 153-9; Archer et al. (1993) *Biochemistry* 32: 1164-71; Daopin et al. (1992) *Science* 257: 369-373; Schlunegger et al. (1992) *Nature* 358: 430-434; Hinck et al. (1996) *Biochemistry* 35: 8517-34; Mittl et al. (1996) *Protein Sci.* 5:1261-71). In addition, members of the superfamily share a similar tertiary structure

Several members of the TGFβ superfamily have been identified which play salient roles during vertebrate development. Dorsalin is expressed preferentially in the dorsal side of the developing chick neural tube (Basler et al. (1993)*Cell* 73:687-702) and plays an important role in neural patterning along the dorsoventral axis. Certain of the bone morphogenetic proteins (BMPs) can induce the formation of ectopic bone and cartilage when implanted under the skin or into muscles (Wozney, J.M. et al. (1988) Sc*ience* 242:1528-1534). In mice, mutations in BMP5 have been found to result in effects on many different skeletal elements, including reduced external ear size and decreased repair of bone fractures in adults (Kingsley (1994) *Genes Dev.* 8:133-146). Besides these effects on bone tissue, BMPs play other roles during normal development. For example, they are expressed in non skeletal tissues (Lyons et al. (1990) *Development* 109:833-844), and injections of BMP4 into developing *Xenopus* embryos promote the formation of ventral/posterior mesoderm (Dale et al (1992) *Development* 115:573-585). A role for TGFβ family members in the development of sex organs has also been described.

In addition, it is known in the art that TGFβ can also promote the growth of other cell types as evidenced by its role in neovascularization and the proliferation of connective tissue cells. Because of these activities, it plays a key role in wound healing (Kovacs, E.J. (1991) *Immunol Today* 12:17-23).

TGFβ therapeutics and proteins

As described above for recombinant polypeptides, TGFβ polypeptides of the invention can comprise essentially any member of the TGFβ superfamily. In preferred embodiments, a TGFβ polypeptide comprises a vertebrate member of the TGFβ

superfamily. TGFβ polypeptides may also comprise members of the TGFβ subfamily. In particularly preferred embodiments, the TGFβ polypeptide comprises a polypeptide that is at least 60%, 70%, 80%, 90%, 95%, or 97-99% identical or homologous to a polypeptide as set forth in any one of SEQ, ID. Nos. 1-10. In a preferred embodiments a TGFβ
5 polypeptide comprises a TGFβ-5 motif as shown in SEQ. ID. Nos. 11-13.

It will be generally appreciated that, under certain circumstances, it may be advantageous to provide homologs of one of the subject TGFβ polypeptides which function in a limited capacity as a TGFβ agonist (mimetic), in order to promote only a subset of the biological activities of the naturally-occurring form of the protein. Thus, specific biological
10 effects can be elicited by treatment with a homolog of limited function, and with fewer side effects relative to treatment with agonists or antagonists which are directed to all of the biological activities of naturally occurring forms of TGFβ proteins.

Homologs of each of the subject TGFβ proteins can be generated by mutagenesis, such as by discrete point mutation(s), or by truncation. For instance, mutation can give rise
15 to homologs which retain substantially the same, or merely a subset, of the biological activity of the TGFβ polypeptide from which it was derived.

In general, polypeptides referred to herein as having an activity (e.g., are "bioactive") of a TGFβ protein are defined as polypeptides which include an amino acid sequence corresponding (e.g., identical or homologous) to all or a portion of the amino acid
20 sequences of a TGFβ proteins or motif shown in any one or more of SEQ ID NOs: 1-11 and which mimic all or a portion of the biological/biochemical activities of a naturally occurring TGFβ protein. Examples of such biological activity include the ability to induce (or otherwise modulate) proliferation and/or differentiation of pancreatic cells in vivo or in vitro.

25 The bioactivity of the subject TGFβ proteins may also include the ability to alter the transcriptional rate of a gene, such as by participating in the transcriptional complexes (activating or inhibiting), e.g., either homo- or hetero-oligomeric in composition, or by altering the composition of a transcriptional complex by modfiying the competency and/or availability of proteins of the complex. The TGFβ gene products may also be involved in
30 regulating post-translational modification of other cellular proteins, e.g., by action of an

intrinsic enzymatic activity, or as a regulatory subunit of an enzyme complex, and/or as a chaperon.

Yet another bioactivity of the subject TGFβ protein is the ability to interact with a TGFβ receptor complex, or a subunit thereof, particularly a receptor complex having a
5 ligand bound thereto.

Other biological activities of the subject TGFβ proteins are described herein or will be reasonably apparent to those skilled in the art. According to the present invention, a polypeptide has biological activity if it is a specific agonist or antagonist of a naturally-occurring form of a vertebrate TGFβ protein.

10 Methods of producing the subject TGFβ polypeptides are well known in the art. For example, a host cell transfected with a nucleic acid vector directing expression of a nucleotide sequence encoding the subject polypeptides can be cultured under appropriate conditions to allow expression of the peptide to occur. The cells may be harvested, lysed and the protein isolated. A cell culture includes host cells, media and other byproducts.
15 Suitable media for cell culture are well known in the art. The recombinant TGFβ polypeptide can be isolated from cell culture medium, host cells, or both using techniques known in the art for purifying proteins including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with antibodies specific for such peptide. In a preferred embodiment, the recombinant
20 TGFβ polypeptide is a fusion protein containing a domain which facilitates its purification, such as GST fusion protein or poly (His) fusion protein.

This invention also pertains to a host cell transfected to express a recombinant form of the subject TGFβ polypeptides. The host cell may be any prokaryotic or eukaryotic cell. Thus, a nucleotide sequence derived from the cloning of vertebrate
25 TGFβ genes, encoding all or a selected portion of the full-length protein, can be used to produce a recombinant form of a vertebrate TGFβ polypeptide via microbial or eukaryotic cellular processes. Ligating the polynucleotide sequence into a gene construct, such as an expression vector, and transforming or transfecting into hosts, either eukaryotic (yeast, avian, insect or mammalian) or prokaryotic (bacterial cells), are standard procedures used in
30 producing other well-known proteins, e.g. MAP kinase, p53, WT1, PTP phosphotases, SRC, and the like. Similar procedures, or modifications thereof, can be employed to

prepare recombinant TGFβ polypeptides by microbial means or tissue-culture technology in accord with the subject invention.

Recombinant TGFβ genes can be produced by ligating nucleic acid encoding a TGFβ protein, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells, or both. Expression vectors for production of recombinant forms of the subject TGFβ polypeptides include plasmids and other vectors. For instance, suitable vectors for the expression of a TGFβ polypeptide include plasmids of the types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*.

A number of vectors exist for the expression of recombinant proteins in yeast. For instance, YEP24, YIP5, YEP51, YEP52, pYES2, and YRP17 are cloning and expression vehicles useful in the introduction of genetic constructs into *S. cerevisiae* (see, for example, Broach *et al.* (1983) in *Experimental Manipulation of Gene Expression*, ed. M. Inouye Academic Press, p. 83, incorporated by reference herein). These vectors can replicate in *E. coli* due the presence of the pBR322 ori, and in *S. cerevisiae* due to the replication determinant of the yeast 2 micron plasmid. In addition, drug resistance markers such as ampicillin can be used. In an illustrative embodiment, a TGFβ polypeptide is produced recombinantly utilizing an expression vector generated by sub-cloning the coding sequence of one of the TGFβ genes encoding the polypeptides represented in SEQ ID Nos: 1-13.

The preferred mammalian expression vectors contain both prokaryotic sequences, to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papillomavirus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. The various methods employed in the preparation of the plasmids and transformation of host organisms are well

known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see *Molecular Cloning A Laboratory Manual*, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989) Chapters 16 and 17.

5 In some instances, it may be desirable to express the recombinant TGFβ polypeptide by the use of a baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the ß-gal containing pBlueBac III).

10 When it is desirable to express only a portion of a TGFβ protein, such as a form lacking a portion of the N-terminus, i.e. a truncation mutant which lacks the signal peptide, it may be necessary to add a start codon (ATG) to the oligonucleotide fragment containing the desired sequence to be expressed. It is well known in the art that a methionine at the N-terminal position can be enzymatically cleaved by the use of the 15 enzyme methionine aminopeptidase (MAP). MAP has been cloned from *E. coli* (Ben-Bassat et al. (1987) *J. Bacteriol.* 169:751-757) and *Salmonella typhimurium* and its *in vitro* activity has been demonstrated on recombinant proteins (Miller et al. (1987) *PNAS 84*:2718-1722). Therefore, removal of an N-terminal methionine, if desired, can be achieved either *in vivo* by expressing *TGFb*-derived polypeptides in a host which produces 20 MAP (e.g., *E. coli* or CM89 or *S. cerevisiae*), or *in vitro* by use of purified MAP (e.g., procedure of Miller et al., *supra*).

The present invention further pertains to methods of producing the subject TGFβ polypeptides. For example, a host cell transfected with a nucleic acid vector directing expression of a nucleotide sequence encoding the subject TGFβ polypeptide can be cultured 25 under appropriate conditions to allow expression of the peptide to occur. The peptide may be secreted and isolated from a mixture of cells and medium containing the recombinant TGFβ polypeptide. Alternatively, the peptide may be retained cytoplasmically by removing the signal peptide sequence from the recombinant TGFβ gene and the cells harvested, lysed and the protein isolated. A cell culture includes host cells, media and other byproducts. 30 Suitable media for cell culture are well known in the art. The recombinant TGFβ polypeptide can be isolated from cell culture medium, host cells, or both using techniques known in the art for purifying proteins including ion-exchange chromatography, gel

filtration chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with antibodies specific for such peptide. In a preferred embodiment, the recombinant TGFβ polypeptide is a fusion protein containing a domain which facilitates its purification, such as a TGFβ/GST fusion protein.

5

In another embodiment, the coding sequences for the polypeptide can be incorporated as a part of a fusion gene including a nucleotide sequence encoding a different polypeptide. This type of expression system can be useful under conditions where it is desirable to produce an immunogenic fragment of a TGFβ protein. For example, the VP6
10 capsid protein of rotavirus can be used as an immunologic carrier protein for portions of the TGFβ polypeptide, either in the monomeric form or in the form of a viral particle. The nucleic acid sequences corresponding to the portion of a subject TGFβ protein to which antibodies are to be raised can be incorporated into a fusion gene construct which includes coding sequences for a late vaccinia virus structural protein to produce a set of recombinant
15 viruses expressing fusion proteins comprising TGFβ epitopes as part of the virion. It has been demonstrated with the use of immunogenic fusion proteins utilizing the Hepatitis B surface antigen fusion proteins that recombinant Hepatitis B virions can be utilized in this role as well. Similarly, chimeric constructs coding for fusion proteins containing a portion of a TGFβ protein and the poliovirus capsid protein can be created to enhance
20 immunogenicity of the set of polypeptide antigens (see, for example, EP Publication No: 0259149; and Evans et al. (1989) *Nature* 339:385; Huang et al. (1988) *J. Virol.* 62:3855; and Schlienger et al. (1992) *J. Virol.* 66:2).

In addition to utilizing fusion proteins to enhance immunogenicity, it is widely appreciated that fusion proteins can also facilitate the expression of proteins, and
25 accordingly, can be used in the expression of the vertebrate TGFβ polypeptides of the present invention. For example, TGFβ polypeptides can be generated as glutathione-S-transferase (GST-fusion) proteins. Such GST-fusion proteins can enable easy purification of the TGFβ polypeptide, as for example by the use of glutathione-derivatized matrices (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al. (N.Y.: John
30 Wiley & Sons, 1991)).

In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired

portion of the recombinant protein, can allow purification of the expressed fusion protein by affinity chromatography using a Ni2+ metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified protein (e.g., see Hochuli et al. (1987) *J. Chromatography* 411:177; and Janknecht et al. *PNAS* 88:8972). Fusions to Green Fluorescent Protein (GFP) and variants thereof (eg. yellow, cyan, red and blue variants known as YFP, CFP, RFP, and BFP respectively).

Techniques for making fusion genes are known to those skilled in the art. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al. John Wiley & Sons: 1992).

TGFβ polypeptides may also be chemically modified to create TGFβ derivatives by forming covalent or aggregate conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives of TGFβ proteins can be prepared by linking the chemical moieties to functional groups on amino acid sidechains of the protein or at the N-terminus or at the C-terminus of the polypeptide.

The present invention also makes available isolated TGFβ polypeptides which are isolated from, or otherwise substantially free of other cellular proteins, especially other signal transduction factors and/or transcription factors which may normally be associated with the TGFβ polypeptide. The term "substantially free of other cellular proteins" (also referred to herein as "contaminating proteins") or "substantially pure or purified preparations" are defined as encompassing preparations of TGFβ polypeptide polypeptides having less than 20% (by dry weight) contaminating protein, and preferably having less than 5% contaminating protein. Functional forms of the subject polypeptides

can be prepared, for the first time, as purified preparations by using a cloned gene as described herein. By "purified", it is meant, when referring to a peptide or DNA or RNA sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules, such as other proteins. The term "purified" as used herein preferably means at least 80% by dry weight, more preferably in the range of 95-99% by weight, and most preferably at least 99.8% by weight, of biological macromolecules of the same type present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 5000, can be present). The term "pure" as used herein preferably has the same numerical limits as "purified" immediately above. "Isolated" and "purified" do not encompass either natural materials in their native state or natural materials that have been separated into components (e.g., in an acrylamide gel) but not obtained either as pure (e.g. lacking contaminating proteins, or chromatography reagents such as denaturing agents and polymers, e.g. acrylamide or agarose) substances or solutions. In preferred embodiments, purified TGFβ preparations will lack any contaminating proteins from the same animal from that TGFβ is normally produced, as can be accomplished by recombinant expression of, for example, a human TGFβ protein in a non-human cell.

Isolated peptidyl portions of TGFβ proteins can be obtained by screening peptides recombinantly produced from the corresponding fragment of the nucleic acid encoding such peptides. In addition, fragments can be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry. For example, a TGFβ polypeptide polypeptide of the present invention may be arbitrarily divided into fragments of desired length with no overlap of the fragments, or preferably divided into overlapping fragments of a desired length. The fragments can be produced (recombinantly or by chemical synthesis) and tested to identify those peptidyl fragments which can function to promote growth or differentiation of pancreatic tissue.

The recombinant TGFβ polypeptides of the present invention also include homologs of the authentic TGFβ proteins, such as versions of those proteins that are resistant to proteolytic cleavage, as for example, due to mutations that alter ubiquitination or other enzymatic targeting associated with the protein.

Modification of the structure of the subject vertebrate TGFβ polypeptides can be for such purposes as enhancing therapeutic or prophylactic efficacy, stability (e.g.,

ex vivo shelf life and resistance to proteolytic degradation *in vivo*), or post-translational modifications (e.g., to alter phosphorylation pattern of protein). Such modified peptides, when designed to retain at least one activity of the naturally-occurring form of the protein, or to produce specific antagonists thereof, are considered functional equivalents of the TGFβ polypeptides described in more detail herein. Such modified peptides can be produced, for instance, by amino acid substitution, deletion, or addition.

For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (i.e. isosteric and/or isoelectric mutations) will not have a major effect on the biological activity of the resulting molecule. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are can be divided into four families: (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine, histidine; (3) nonpolar = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar = glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. In similar fashion, the amino acid repertoire can be grouped as (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine histidine, (3) aliphatic = glycine, alanine, valine, leucine, isoleucine, serine, threonine, with serine and threonine optionally be grouped separately as aliphatic-hydroxyl; (4) aromatic = phenylalanine, tyrosine, tryptophan; (5) amide = asparagine, glutamine; and (6) sulfur - containing = cysteine and methionine. (see, for example, *Biochemistry*, 2nd ed., Ed. by L. Stryer, WH Freeman and Co.: 1981). Whether a change in the amino acid sequence of a peptide results in a functional *TGFb* homolog (e.g. functional in the sense that the resulting polypeptide mimics or antagonizes the wild-type form) can be readily determined by assessing the ability of the variant peptide to produce a response in cells in a fashion similar to the wild-type protein, or competitively inhibit such a response. Polypeptides in which more than one replacement has taken place can readily be tested in the same manner.

This invention further contemplates a method for generating sets of combinatorial mutants of the subject TGFβ proteins as well as truncation mutants, and is especially useful for identifying potential variant sequences (e.g. homologs) that are functional in modulating signal transduction from a TGFβ receptor. The purpose of screening such combinatorial libraries is to generate, for example, novel TGFβ homologs

which can affect, eg. pancreatic cell proliferation and/or differentiation. Combinatorially-derived homologs can be generated to have an increased potency relative to a naturally occurring form of the protein.

In one aspect of this method, the amino acid sequences for a population of TGFβ homologs or other related proteins are aligned, preferably to promote the highest homology possible (Figure 12). Such a population of variants can include, for example, TGFβ homologs from one or more species. Amino acids which appear at each position of the aligned sequences are selected to create a degenerate set of combinatorial sequences. In a preferred embodiment, the variegated library of TGFβ variants is generated by combinatorial mutagenesis at the nucleic acid level, and is encoded by a variegated gene library. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential TGFβ sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g. for phage display) containing the set of TGFβ sequences therein.

In an illustrative embodiment, alignment of the TGFβ -motifs for the Xenopus TGFβ-5 and human TGFβ -1, -2 and -3 can be used to produce a degenerate set of polypeptides that share features distinctive of TGFβ proteins but also properties unique to TGFβ -5 polypeptides. For example, TGFβ -5 motifs are shown in Figure 11 (SEQ. ID. Nos. 11-13). A set of 9 conserved cysteine residues at fixed positions are sufficient to define a subset of TGFβ proteins to which TGFβ -5 belongs. The additional constraint of, for example, requiring a charged amino acid at positions shown in SEQ. ID. No. 12 creates a motif that is specific for TGFβ -5 and excludes all other currently known TGFβ proteins.

There are many ways by which libraries of potential TGFβ homologs can be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be carried out in an automatic DNA synthesizer, and the synthetic genes then ligated into an appropriate expression vector. The purpose of a degenerate set of genes is to provide, in one mixture, all of the sequences encoding the desired set of potential TGFβ sequences. The synthesis of degenerate oligonucleotides is well known in the art (see for example, Narang, SA (1983) *Tetrahedron* 39:3; Itakura et al. (1981) *Recombinant DNA, Proc 3rd Cleveland Sympos. Macromolecules*, ed. AG Walton, Amsterdam: Elsevier pp273-289; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1056; Ike et al. (1983) *Nucleic Acid Res.* 11:477. Such techniques have been employed

in the directed evolution of other proteins (see, for example, Scott et al. (1990) *Science* 249:386-390; Roberts et al. (1992) *PNAS* 89:2429-2433; Devlin et al. (1990) *Science* 249: 404-406; Cwirla et al. (1990) *PNAS* 87: 6378-6382; as well as U.S. Patents Nos. 5,223,409, 5,198,346, and 5,096,815).

5 Likewise, a library of coding sequence fragments can be provided for a TGFβ clone in order to generate a variegated population of TGFβ fragments for screening and subsequent selection of bioactive fragments. A variety of techniques are known in the art for generating such libraries, including chemical synthesis. In one embodiment, a library of coding sequence fragments can be generated by (i) treating a double stranded 10 PCR fragment of a TGFβ coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule; (ii) denaturing the double stranded DNA; (iii) renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products; (iv) removing single stranded portions from reformed duplexes by treatment with S1 nuclease; and (v) ligating the resulting fragment library into 15 an expression vector. By this exemplary method, an expression library can be derived which codes for N-terminal, C-terminal and internal fragments of various sizes.

A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a certain property. Such techniques will be generally 20 adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of TGFβ homologs. The most widely used techniques for screening large gene libraries typically comprises cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates 25 relatively easy isolation of the vector encoding the gene whose product was detected. Each of the illustrative assays described below are amenable to high through-put analysis as necessary to screen large numbers of degenerate TGFβ sequences created by combinatorial mutagenesis techniques.

Combinatorial mutagenesis has a potential to generate very large libraries of mutant 30 proteins, e.g., in the order of 10^{26} molecules. Combinatorial libraries of this size may be technically challenging to screen even with high throughput screening assays. To overcome this problem, a new technique has been developed recently, recrusive ensemble

mutagenesis (REM), which allows one to avoid the very high proportion of non-functional proteins in a random library and simply enhances the frequency of functional proteins, thus decreasing the complexity required to achieve a useful sampling of sequence space. REM is an algorithm which enhances the frequency of functional mutants in a library when an
5 appropriate selection or screening method is employed (Arkin and Yourvan, 1992, *PNAS USA* 89:7811-7815; Yourvan et al., 1992, *Parallel Problem Solving from Nature*, 2., In Maenner and Manderick, eds., Elsevir Publishing Co., Amsterdam, pp. 401-410; Delgrave et al., 1993, *Protein Engineering* 6(3):327-331).

Mutagenic techniques as described above are also useful to map the
10 determinants of the TGFβ proteins which participate in protein-protein interactions involved in, for example, binding of the subject TGFβ polypeptide to proteins which may function upstream (including both activators and repressors of its activity) or to proteins or nucleic acids which may function downstream of the TGFβ polypeptide, whether they are positively or negatively regulated by it. To illustrate, the critical residues of a subject TGFβ
15 polypeptide which are involved in molecular recognition of an upstream or downstream TGFβ component can be determined and used to generate TGFβ -derived peptidomimetics which competitively inhibit binding of the authentic TGFβ protein with that moiety. By employing, for example, scanning mutagenesis to map the amino acid residues of each of the subject TGFβ proteins which are involved in binding other extracellular proteins,
20 peptidomimetic compounds can be generated which mimic those residues of the TGFβ protein which facilitate the interaction. Such mimetics may then be used to interfere with the normal function of a TGFβ protein. For instance, non-hydrolyzable peptide analogs of such residues can be generated using benzodiazepine (e.g., see Freidinger et al. in *Peptides: Chemistry and Biology*, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988),
25 azepine (e.g., see Huffman et al. in *Peptides: Chemistry and Biology*, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), substituted gamma lactam rings (Garvey et al. in *Peptides: Chemistry and Biology*, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), keto-methylene pseudopeptides (Ewenson et al. (1986) *J Med Chem* 29:295; and Ewenson et al. in *Peptides: Structure and Function* (Proceedings of the 9th
30 American Peptide Symposium) Pierce Chemical Co. Rockland, IL, 1985), β-turn dipeptide cores (Nagai et al. (1985) *Tetrahedron Lett* 26:647; and Sato et al. (1986) *J Chem Soc Perkin Trans* 1:1231), and β-aminoalcohols (Gordon et al. (1985) *Biochem Biophys Res*

*Commun*126:419; and Dann et al. (1986) *Biochem Biophys Res Commun* 134:71). Furthermore, by making available purified and recombinant TGFβ polypeptides, the present invention facilitates the development of assays which can be used to screen for molecules that may be useful as TGFβ therapeutics, including TGFβ homologs, which are either agonists or antagonists of the normal cellular function of the subject TGFβ polypeptides, or of their role in the pathogenesis of cellular differentiation and/or proliferation and disorders related thereto. In one embodiment, the assay evaluates the ability of a compound to modulate binding between a TGFβ polypeptide and a molecule, be it protein or DNA, that interacts either upstream or downstream of the TGFβ polypeptide in the TGFβ signaling pathway. For instance, the assay can be used to identify compounds which either inhibit or potentiate the interaction of a TGFβ polypeptide with a TGFβ receptor complex or subunit thereof. A variety of assay formats will suffice and, in light of the present inventions, will be comprehended by a skilled artisan.

In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored in the *in vitro* system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity with upstream or downstream elements. Accordingly, in an exemplary screening assay of the present invention, the compound of interest is contacted with proteins which may function upstream (including both activators and repressors of its activity) or to proteins or nucleic acids which may function downstream of the TGFβ polypeptide, whether they are positively or negatively regulated by it. To the mixture of the compound and the upstream or downstream element is then added a composition containing a TGFβ polypeptide. Detection and quantification of complexes of TGFβ with it's upstream or downstream elements provide a means for determining a compound's efficacy at inhibiting (or potentiating) complex formation between TGFβ and the TGFβ -binding elements. The efficacy of the compound can be assessed by generating dose response

curves from data obtained using various concentrations of the test compound. Moreover, a control assay can also be performed to provide a baseline for comparison. In the control assay, isolated and purified TGFβ polypeptide is added to a composition containing the TGFβ -binding element, and the formation of a complex is quantitated in the absence of the test compound.

Complex formation between the TGFβ polypeptide and a TGFβ binding element may be detected by a variety of techniques. Modulation of the formation of complexes can be quantitated using, for example, detectably labeled proteins such as radiolabeled, fluorescently labeled, or enzymatically labeled TGFβ polypeptides, by immunoassay, or by chromatographic detection.

Typically, it will be desirable to immobilize either TGFβ or its binding protein to facilitate separation of complexes from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of TGFβ to an upstream or downstream element, in the presence and absence of a candidate agent, can be accomplished in any vessel suitable for containing the reactants. Examples include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows the protein to be bound to a matrix. For example, glutathione-S-transferase/ TGFβ (GST/TGFβ) fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the cell lysates, e.g. an ^{35}S-labeled, and the test compound, and the mixture incubated under conditions conducive to complex formation, e.g. at physiological conditions for salt and pH, though slightly more stringent conditions may be desired. Following incubation, the beads are washed to remove any unbound label, and the matrix immobilized and radiolabel determined directly (e.g. beads placed in scintilant), or in the supernatant after the complexes are subsequently dissociated. Alternatively, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of TGFβ -binding protein found in the bead fraction quantitated from the gel using standard electrophoretic techniques such as described in the appended examples. Other techniques for immobilizing proteins on matrices are also available for use in the subject assay and known in the art.

For processes which rely on immunodetection for quantitating one of the proteins trapped in the complex, antibodies against the protein, such as anti-TGFβ

antibodies, can be used. Alternatively, the protein to be detected in the complex can be "epitope tagged" in the form of a fusion protein which includes, in addition to the TGFβ sequence, a second polypeptide for which antibodies are readily available (e.g. from commercial sources). For instance, the GST fusion proteins described above can also be used for quantification of binding using antibodies against the GST moiety. Other useful epitope tags include myc-epitopes (e.g., see Ellison et al. (1991) J Biol Chem 266:21150-21157) which includes a 10-residue sequence from c-myc, as well as the pFLAG system (International Biotechnologies, Inc.) or the pEZZ-protein A system (Pharamacia, NJ).

In addition to cell-free assays, such as described above, the readily available source of vertebrate TGFβ proteins provided by the present invention also facilitates the generation of cell-based assays for identifying small molecule agonists/antagonists and the like. Cells, such as pancreatic cells, which are sensitive to TGFβ -mediated induction by a TGFβ can be caused to overexpress a recombinant TGFβ protein in the presence and absence of a test agent of interest, with the assay scoring for modulation in TGFβ inductive responses by the target cell mediated by the test agent. As with the cell-free assays, agents which produce a statistically significant change in TGFβ -dependent induction (either inhibition or potentiation) can be identified.

Furthermore, each of the assay systems set out above can be generated in a "differential" format. That is, the assay format can provide information regarding specificity as well as potency. For instance, side-by-side comparison of a test compound's effect on different TGFβ proteins can provide information on selectivity, and permit the identification of compounds which selectively modulate the bioactivity of only a subset of the TGFβ family.

Cells and Treatments

Another aspect of the present invention relates to a method of inducing and/or maintaining a differentiated state, enhancing survival, and/or promoting proliferation of a cell responsive to a TGF-β factor, by contacting the cells with a TGFβ polypeptide or a TGFβ therapeutic. For instance, it is contemplated by the invention that, in light of the present finding that TGFβ proteins stimulate growth and differentiation of cells such as the pancreatic cells, the subject method could be used to generate and/or maintain tissue such as

the pancreatic tissue both *in vitro* and *in vivo*. A "TGFβ therapeutic," can be, as appropriate, any of the preparations described above, including isolated polypeptides, gene therapy constructs, antisense molecules, peptidomimetics or agents identified in the drug assays provided herein.

5 There are a wide variety of pathological conditions for which TGFβ therapeutics and proteins of the present invention can be used in treatment. For instance, such agents can provide therapeutic benefits where the general strategy is to increase proliferation or maintenance of pancreatic tissue. Diseases that might benefit from this methodology include, but are not limited to forms of diabetes.

10 In addition to proliferative disorders, the present invention contemplates the use of TGFβ therapeutics for the treatment of differentiative disorders which result from, for example, de-differentiation of pancreatic tissue which may (optionally) be accompanied by abortive reentry into mitosis, e.g. apoptosis. Such degenerative disorders also include forms of diabetes.

15 It will also be apparent that, by transient use of modulators of TGFβ pathways, *in vivo* reformation of tissue can be accomplished, e.g. in the development and maintenance of organs. By controlling the proliferative and differentiative potential for different cells, the subject gene constructs can be used to reform injured tissue, or to improve grafting and morphology of transplanted tissue. For instance, TGFβ agonists can be employed in a to
20 regulate different stages of organ repair after physical, chemical or pathological insult. For example, such regimens can be utilized in repair of partial surgical removal of the pancreas.

For example, the present method is applicable to cell culture techniques. *In vitro* pancreatic cells and tissues, such as duct tissue can be induced to proliferate. In such embodiments of the subject method, the cultured cells can be contacted with TGFβ
25 therapeutic or protein. The TGFβ therapeutic or protein can be used alone, or can be used in combination with other factors which act to more particularly enhance a particular differentiation fate or activity of the pancreatic tissue. For example the PYY factor is known to assist in activation of pancreatic cells for insulin production.

In the later instance, a TGFβ therapeutic might be viewed as ensuring that the
30 treated cell has achieved a particular phenotypic state such that the cell is poised along a certain developmental pathway so as to be properly induced upon contact with a secondary neurotrophic factor. In similar fashion, even relatively undifferentiated stem cells or

primitive neuroblasts can be maintained in culture and caused to differentiate by treatment with TGFβ therapeutics. Exemplary primitive cell cultures comprise cells harvested from the neural plate or neural tube of an embryo even before much overt differentiation has occurred.

5 Yet another aspect of the present invention concerns the application of TGFβ therapeutics to modulating morphogenic signals involved in other vertebrate organogenic pathways in addition to neuronal differentiation, e.g., to TGF-β roles in both mesodermal and ectodermal differentiation processes. Thus, it is contemplated by the invention that compositions comprising TGFβ therapeutics can also be utilized for both cell culture and 10 therapeutic methods involving generation and maintenance of non-neuronal tissue.

In one embodiment, the present invention makes use of the discovery that TGFβ proteins are likely to be involved in controlling the development and formation of the digestive tract, liver, pancreas, lungs, and other organs which derive from the primitive gut. As described in the Examples below, TGFβ proteins a presumptively involved in cellular 15 activity in response to TGF-β inductive signals. Accordingly, TGFβ agonists and/or antagonists can be employed in the development and maintenance of an artificial liver which can have multiple metabolic functions of a normal liver. In an exemplary embodiment, TGFβ therapeutics can be used to induce and/or maintain differentiation of digestive tube stem cells to form hepatocyte cultures which can be used to populate 20 extracellular matrices, or which can be encapsulated in biocompatible polymers, to form both implantable and extracorporeal artificial livers.

In another embodiment, compositions of TGFβ therapeutics can be utilized in conjunction with transplantation of such artificial livers, as well as embryonic liver structures, to promote intraperitoneal implantation, vascularization, and *in vivo* 25 differentiation and maintenance of the engrafted liver tissue.

Similar utilization of TGFβ therapeutics are contemplated in the generation and maintenance of pancreatic cultures and artificial pancreatic tissues and organs.

In one embodiment, TGFβ therapeutics are contacted with small cross-sections of pancreatic ductal tissue under conditions sufficient to induce outgrowth of the tissue thereby 30 forming new pancreatic tissue. In another embodiment, the TGFβ therapeutics can be contacted with micro-organ explant tissue, e.g., ductal tissue explants. Ductal tissue explants preferably are derived with dimensions that allow the explanted tissue to maintain

its microarchitecture and biological function for prolonged periods of time in culture, e.g., the dimensions of the explant preserve the normal tissue architecture and at least a portion of the normal tissue function that is present in vivo. Such tissue explants can be maintained, for instance, in minimal culture media for extended periods of time (e.g., for 21 days or longer) and can be contacted with different factors, including TGFβ therapeutics. Carefully defined conditions can be acquired in the culture so as selectively activate discrete populations of cells in the tissue explant. Certain cells or tissues can be subsequently isolated from the explant, based upon its response to the addition of TGFβ therapeutics or other growth factors to the culture.

The micro-organ cultures used as substrates for TGFβ therapeutics or TGFβ protein-producing cells according to the invention preserve the normal tissue architecture that is present in vivo, e.g., the original epithelial-mesenchymal organization. In preferred embodiments, the populations of cells of the ductal explants are grouped in a manner that preserves the natural affinity of one cell to another, e.g., to preserve layers of different cells present in explant. Such an association facilitates intercellular communication. Many types of communication take place among animal cells. This is particularly important in differentiating cells where induction is defined as the interaction between one (inducing) and another (responding) tissue or cell, as a result of which the responding cells undergo a change in differentiation. Moreover, inductive interactions occur in embryonic and adult cells and can act to establish and maintain morphogenetic patterns and also induce differentiation (Gurdon (1992) Cell 68: 185-199). Exemplary micro-organ cultures which can be used in the method of the invention are described in the Examples and include epithelial and mesenchymal cells grouped in a manner that includes a plurality of layers so as to preserve the natural affinity and interaction of one cell to another in and between each layer.

In another embodiment, the ductal explants and/or pancreatic cells or tissue can be cultured on feeder layers, e.g., layers of feeder cells which secrete TGFβ protein or polymeric layers containing TGFβ protein. Natural or recombinantly engineered cells can be provided as feeder layers to the instant cultures.

Methods of measuring cell proliferation are well known in the art and most commonly include determining DNA synthesis characteristic of cell replication. There are numerous methods in the art for measuring DNA synthesis, any of which may be used according to the invention. In an embodiment of the invention, DNA synthesis has been

determined using a radioactive label (^{3}H-thymidine) or labeled nucleotide analogues (BrdU) for detection by immunofluorescence.

In another preferred embodiment, the subject TGFβ protein producing cells can be implanted into one of a number of regeneration models used in the art, e.g., partial
5 pancreatectomy or streptozocin treatment of a host animal.

Accordingly, another aspect of the present invention pertains to the pancreatic tissue in which growth has been induced according to the invention.

Yet another aspect of the present invention concerns cellular compositions which include, as a cellular component, substantially pure preparations of the subject TGFβ
10 protein producing cells. Cellular compositions of the present invention include not only substantially pure populations of the TGFβ protein producing cells, but can also include cell culture components, e.g., culture media including amino acids, metals, coenzyme factors, as well as small populations of non-TGFβ protein producing cells, e.g., some of which may arise by subsequent differentiation of the cells of the invention. Furthermore, other non-
15 cellular components include those which render the cellular component suitable for support under particular circumstances, e.g., implantation, e.g., continuous culture.

In yet another embodiment, the invention features a pharmaceutical composition including as the cellular component, a substantially pure population of TGFβ protein producing cells, which cells are capable of inducing outgrowth of pancreatic tissue in a
20 culture medium.

In general, the pancreatic ductal cells in which outgrowth is induced by TGFβ protein or TGFβ protein-producing cells will be of mammalian origin, e.g., cells isolated from a primate such as a human, from a miniature swine, or from a transgenic mammal, or are the cell culture progeny of such cells. In one embodiment, pancreatic ductal tissue is
25 isolated from a patient and subjected to the present method in order to provide a resulting culture of pancreatic cells or tissue (or differentiated cells derived therefrom). The isolated cells or tissue then can be transplanted back into the initial donor patient or into a second host patient.

In another aspect, the invention features, a method for screening a compound for
30 ability to modulate one of growth, proliferation, and/or differentiation of progenitor cells obtained by the subject method, including: (i) establishing an isolated population of pancreatic progenitor cells; (ii) contacting the population of cells with a test compound; and (iii) detecting one of growth, proliferation, and/or differentiation of the progenitor cells in

the population, wherein a statistically significant change in the extent of one of growth, proliferation, and/or differentiation in the presence of the test compound relative to the extent of one of growth, proliferation, and/or differentiation in the absence of the test compound indicates the ability of the test compound to modulate one of the growth, 5 proliferation, and/or differentiation.

In another aspect, the invention features, a method for treating a disorder characterized by insufficient insulin activity, in a subject, including introducing into the subject a pharmaceutical composition including pancreatic cells derived by the subject method, or differentiated cells arising therefrom, and a pharmaceutically acceptable carrier.

10 In a preferred embodiment the subject is a mammal, e.g., a primate, e.g., a human.

In another preferred embodiment the disorder is an insulin dependent diabetes, e.g., type I diabetes.

In yet another preferred embodiment, the pancreatic duct cells are induced to differentiate into pancreatic islet cells, e.g., β islet cells, α islet cells, δ islet cells, or ϕ islet 15 cells, subsequent to being introduced into the subject. Preferably, the pancreatic progenitors cells are induced to differentiate into pancreatic islet, e.g., β islet cells, α islet cells, δ islet cells, or ϕ islet cells, in culture prior to introduction into the subject.

As common methods of administering the cells of the present invention to subjects, particularly human subjects, which are described in detail herein, include injection or 20 implantation of the cells into target sites in the subjects, the cells of the invention can be inserted into a delivery device which facilitates introduction by, injection or implantation, of the cells into the subjects. Such delivery devices include tubes, e.g., catheters, for injecting cells and fluids into the body of a recipient subject. In a preferred embodiment, the tubes additionally have a needle, e.g., a syringe, through which the cells of the invention 25 can be introduced into the subject at a desired location. The TGFβ protein producing cells of the invention can be inserted into such a delivery device, e.g., a syringe, in different forms. For example, the cells can be suspended in a solution or embedded in a support matrix when contained in such a delivery device. As used herein, the term "solution" includes a pharmaceutically acceptable carrier or diluent in which the cells of the invention 30 remain viable.

Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. The solution is preferably sterile and fluid to the extent that easy

syringability exists. Preferably, the solution is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi through the use of, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. Solutions of the invention can be prepared by incorporating progenitor cells as described herein in a pharmaceutically acceptable carrier or diluent and, as required, other ingredients enumerated above, followed by filtered sterilization.

Support matrices in which the cells can be incorporated or embedded include matrices which are recipient-compatible and which degrade into products which are not harmful to the recipient. Natural and/or synthetic biodegradable matrices are examples of such matrices. Natural biodegradable matrices include plasma clots, e.g., derived from a mammal, and collagen matrices. Synthetic biodegradable matrices include synthetic polymers such as polyanhydrides, polyorthoesters, and polylactic acid. Other examples of synthetic polymers and methods of incorporating or embedding cells into these matrices are known in the art. See e.g., U.S. Patent No. 4,298,002 and U.S. Patent No. 5,308,701. These matrices provide support and protection for the fragile progenitor cells in vivo and are, therefore, the preferred form in which the progenitor cells are introduced into the recipient subjects.

The present invention also provides substantially pure embryonic pancreatic TGFβ protein producing cells which can be used therapeutically for treatment of various disorders associated with insufficient functioning of the pancreas or liver. For instance, the cells can be used to produce populations of pancreatic tissue or differentiated pancreatic cells for repair subsequent to partial pancreatectomy, e.g., excision of a portion of the pancreas. Likewise, such cell populations can be used to regenerate or replace pancreatic tissue loss due to, pancreatolysis, e.g., destruction of pancreatic tissue, such as pancreatitis, e.g., a condition due to autolysis of pancreatic tissue caused by escape of enzymes into the substance.

In an exemplary embodiment, TGFβ protein or TGFβ protein-producing cells can be used to induce tissue growth in pancreatic tissue of a patient suffering from any insulin-deficiency. The tissue then can be activated using PYY, for example, and the activated tissue then can be implanted in the donor patient. Each year, over 728,000 new cases of diabetes are diagnosed and 150,000 Americans die from the disease and its complications; the total yearly cost in the United States is over 20 billion dollars (Langer et al. (1993)

Science 260:920-926). Diabetes is characterized by pancreatic islet destruction or dysfunction leading to loss of glucose control. Diabetes mellitus is a metabolic disorder defined by the presence of chronically elevated levels of blood glucose (hyperglycemia). Insulin-dependent (Type 1) diabetes mellitus ("IDDM") results from an autoimmune-mediated destruction of the pancreatic β-cells with consequent loss of insulin production, which results in hyperglycemia. Type 1 diabetics require insulin replacement therapy to ensure survival. Non-insulin-dependent (Type 2) diabetes mellitus ("NIDDM") is initially characterized by hyperglycemia in the presence of higher-than-normal levels of plasma insulin (hyperinsulinemia). In Type 2 diabetes, tissue processes which control carbohydrate metabolism are believed to have decreased sensitivity to insulin. Progression of the Type 2 diabetic state is associated with increasing concentrations of blood glucose, and coupled with a relative decrease in the rate of glucose-induced insulin secretion.

The primary aim of treatment in both forms of diabetes mellitus is the same, namely, the reduction of blood glucose levels to as near normal as possible. Treatment of Type 1 diabetes involves administration of replacement doses of insulin. In contrast, treatment of Type 2 diabetes frequently does not require administration of insulin. For example, initial therapy of Type 2 diabetes may be based on diet and lifestyle changes augmented by therapy with oral hypoglycemic agents such as sulfonylurea. Insulin therapy may be required, however, especially in the later stages of the disease, to produce control of hyperglycemia in an attempt to minimize complications of the disease, which may arise from islet exhaustion.

More recently, tissue-engineering approaches to treatment have focused on transplanting healthy pancreatic islets, usually encapsulated in a membrane to avoid immune rejection. Three general approaches have been tested in animal models. In the first, a tubular membrane is coiled in a housing that contained islets. The membrane is connected to a polymer graph that in turn connects the device to blood vessels. By manipulation of the membrane permeability, so as to allow free diffusion of glucose and insulin back and forth through the membrane, yet block passage of antibodies and lymphocytes, normoglycemia was maintained in pancreatectomized animals treated with this device (Sullivan et al. (1991) Science 252:718).

In a second approach, hollow fibers containing islet cells were immobilized in the polysaccharide alginate. When the device was place intraperitoneally in diabetic animals,

blood glucose levels were lowered and good tissue compatibility was observed (Lacey et al. (1991) Science 254:1782).

Finally, islets have been placed in microcapsules composed of alginate or polyacrylates. In some cases, animals treated with these microcapsules maintained normoglycemia for over two years (Lim et al. (1980) Science 210:908; O'Shea et al. (1984) Biochim. Biochys. Acta. 840:133; Sugamori et al. (1989) Trans. Am. Soc. Artif. Intern. Organs 35:791; Levesque et al. (1992) Endocrinology 130:644; and Lim et al. (1992) Transplantation 53:1180). However, all of these transplantation strategies require a large, reliable source of donor islets.

TGFβ protein or TGFβ protein producing cells of the invention can be used for treatment of diabetes because they have the ability to induce growth of cells of pancreatic lineage, e.g., β islet cells. The TGFβ protein producing cells of the invention can be cultured in vitro and used to induce pancreatic cells, such as duct tissue cells, to differentiate into mature pancreatic cells, or they can undergo differentiation in vivo once introduced into a subject. Many methods for encapsulating cells are known in the art. For example, a source of β islet cells producing insulin is encapsulated in implantable hollow fibers. Such fibers can be pre-spun and subsequently loaded with the β islet cells (Aebischer et al. U.S. Patent No. 4,892,538; Aebischer et al. U.S. Patent No. 5,106,627; Hoffman et al. (1990) Expt. Neurobiol. 110:39-44; Jaeger et al. (1990) Prog. Brain Res. 82:41-46; and Aebischer et al. (1991) J. Biomech. Eng. 113:178-183), or can be co-extruded with a polymer which acts to form a polymeric coat about the β islet cells (Lim U.S. Patent No. 4,391,909; Sefton U.S. Patent No. 4,353,888; Sugamori et al. (1989) Trans. Am. Artif. Intern. Organs 35:791-799; Sefton et al. (1987) Biotehnol. Bioeng. 29:1135-1143; and Aebischer et al. (1991) Biomaterials 12:50-55).

Moreover, in addition to providing a source of implantable cells, either in the form of a cell population or the differentiated progeny thereof, the subject cells can be used to produce cultures of pancreatic cells for production and purification of secreted TGFβ protein and other factors. For instance, cultured cells can be provided as a source of insulin. Likewise, exocrine cultures can be provided as a source for pancreatin.

Yet another aspect of the present invention provides methods for screening various compounds for their ability to modulate growth, proliferation or differentiation of distinct progenitor cell populations from pancreatic duct tissue. A micro-organ explant that closely mimics the properties of a given set of tissue in vivo would have utility in screening assays

in which compounds could be tested for their ability to modulate one of growth, proliferation or differentiation of progenitor cells in such tissue. Requirements of a reproducible model for screening might include consistency in the micro-architecture, e.g. epithelial-mesenchymal interactions, and nutritional environment in vitro, as well as prolonged viability and proliferation of cultures beyond 24 hours to observe threshold effects of compounds being screened. This level of consistency cannot be achieved in the presence of undefined media supplements such as sera or tissue extracts that vary between batches and cannot be adequately controlled. The dependence of a model on external growth supplements such as growth factors is also undesirable as growth factors or hormones may be included among the compounds to be tested.

In an illustrative embodiment, ductal explants that maintain their microarchitecture in culture, e.g., they preserve the normal epithelial-mesenchymal architecture that is present in vivo, can be used to screen various compounds or natural products. Such explants can be maintained in minimal culture media for extended periods of time (e.g., for 21 days or longer) and can be contacted with any compound, e.g., small molecule or natural product, e.g., TGFβ protein or other growth factor, to determine the effect of such compound on one of cellular growth, proliferation or differentiation of cells in the explant. Detection and quantification of growth, proliferation or differentiation of these cells in response to a given compound provides a means for determining the compound's efficacy at inducing one of the growth, proliferation or differentiation in a given ductal explant. Methods of measuring cell proliferation are well known in the art and most commonly include determining DNA synthesis characteristic of cell replication. There are numerous methods in the art for measuring DNA synthesis, any of which may be used according to the invention. In an embodiment of the invention, DNA synthesis has been determined using a radioactive label (^{3}H-thymidine) or labeled nucleotide analogues (BrdU) for detection by immunofluorescence. The efficacy of the compound can be assessed by generating dose response curves from data obtained using various concentrations of the compound. A control assay can also be performed to provide a baseline for comparison. Identification of the progenitor cell population(s) amplified in response to a given test agent can be carried out according to such phenotyping as described above.

Yet another aspect of the present invention concerns the therapeutic application of a TGFβ therapeutic to enhance survival of neurons and other neuronal cells in both the central nervous system and the peripheral nervous system. The ability of TGF-β factors to

regulate neuronal differentiation during development of the nervous system and also in the adult state indicates that certain of the TGFβ proteins can be reasonably expected to participate in control of adult neurons with regard to maintenance, functional performance, and aging of normal cells; repair and regeneration processes in chemically or mechanically lesioned cells; and prevention of degeneration and premature death which result from loss of differentiation in certain pathological conditions. In light of this understanding, the present invention specifically contemplates applications of the subject method to the treatment of (prevention and/or reduction of the severity of) neurological conditions deriving from: (i) acute, subacute, or chronic injury to the nervous system, including traumatic injury, chemical injury, vasal injury and deficits (such as the ischemia resulting from stroke), together with infectious/inflammatory and tumor-induced injury; (ii) aging of the nervous system including Alzheimer's disease; (iii) chronic neurodegenerative diseases of the nervous system, including Parkinson's disease, Huntington's chorea, amylotrophic lateral sclerosis and the like, as well as spinocerebellar degenerations; and (iv) chronic immunological diseases of the nervous system or affecting the nervous system, including multiple sclerosis.

Methods of introduction of exogenous TGFβ polypeptides at the site of treatment include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, oral, and intranasal. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

Methods of introduction may also be provided by rechargable or biodegradable devices, particularly where gradients of TGFβ concentrations in a tissue is desired.

In yet another embodiment of the present invention, the pharmaceutical TGFβ polypeptide can be administered as part of a combinatorial therapy with other agents. For example, the combinatorial therapy can include a TGFβ protein with at least one trophic factor. Exemplary trophic factors include insulin like growth factor, nerve growth factor, cilliary neurotrophic growth factor, schwanoma-derived growth factor, glial growth factor, stiatal-derived neuronotrophic factor, platelet-derived growth factor, and scatter factor (HGF-SF). In another embodiment of the present invention, the TGFβ therapeutics are

administered in conjunction with other agents, such as one or more of the BMP's, which may synergize with the TGFβ therapeutic and reduce the dosage required to achieve a beneficial effect.

5 *Exemplary pharmaceutical preparations of TGFβ therapeutics*

The TGFβ therapeutic formulations used in the method of the invention are most preferably applied in the form of appropriate compositions. As appropriate compositions there may be cited all compositions usually employed for systemically or topically
10 administering drugs. The pharmaceutically acceptable carrier should be substantially inert, so as not to act with the active component. Suitable inert carriers include water, alcohol polyethylene glycol, mineral oil or petroleum gel, propylene glycol and the like.

To prepare the pharmaceutical compositions of this invention, an effective amount
15 of the particular TGFβ therapeutic as the active ingredient is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of preparation desired for administration. These pharmaceutical compositions are desirable in unitary dosage form suitable, particularly, for administration orally, rectally, percutaneously, or by parenteral injection. For example, in
20 preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed such as, for example, water, glycols, oils, alcohols and the like in the case of oral liquid preparations such as suspensions, syrups, elixirs and solutions; or solid carriers such as starches, sugars, kaolin, lubricants, binders, disintegrating agents and the like in the case of powders, pills, capsules, and tablets. Because of their ease in administration, tablets
25 and capsules represents the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. For parenteral compositions, the carrier will usually comprise sterile water, at least in large part, though other ingredients, for example, to aid solubility, may be included. Injectable solutions, for example, may be prepared in which the carrier comprises saline solution, glucose solution or a mixture of
30 saline and glucose solution. Injectable suspensions may also be prepared in which case appropriate liquid carriers, suspending agents and the like may be employed. Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations. In the compositons suitable for percutaneous administration, the carrier

optionally comprises a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives of any nature in minor proportions, which additives do not introduce a significant deleterious effect on the skin.

In addition to the direct topical application of the preparations they can be topically administered by other methods, for example, encapsulated in a temperature and/or pressure sensitive matrix or in film or solid carrier which is soluble in body fluids and the like for subsequent release, preferably sustained-release of the active component.

As appropriate compositions for topical application there may be cited all compositions usually employed for topically administering therapeuitcs, e.g., creams, gellies, dressings, shampoos, tinctures, pastes, ointments, salves, powders, liquid or semiliquid formulation and the like. Application of said compositions may be by aerosol e.g. with a propellent such as nitrogen carbon dioxide, a freon, or without a propellent such as a pump spray, drops, lotions, or a semisolid such as a thickened composition which can be applied by a swab. In particular compositions, semisolid compositions such as salves, creams, pastes, gellies, ointments and the like will conveniently be used.

It is especially advantageous to formulate the subject compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used in the specification and claims herein refers to physically discreate units suitable as unitary dosages, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Examples of such dosage unit forms are tablets (including scored or coated tablets), capsules, pills, powders packets, wafers, injectable solutions or suspensions, teaspoonfuls, tablespoonfuls and the like, and segregated multiples thereof.

The pharmaceutical preparations of the present invention can be used, as stated above, for the many applications whcih can be considered cosmetic uses. Cosmetic compositions known in the art, preferably hypoallergic and pH controlled are especially preferred, and include toilet waters, packs, lotions, skin milks or milky lotions. The preparations contain, besides the TGFβ therapeutic, components usually employed in such preparations. Examples of such components are oils, fats, waxes, surfactants, humectants, thickening agents, antioxidants, viscosity stabilizers, chelating agents, buffers, preservatives, perfumes, dyestuffs, lower alkanols, and the like. If desired, further ingredients may be incorporated in the compositions, e.g. antiinflammatory agents,

antibacterials, antifungals, disinfectants, vitamins, sunscreens, antibiotics, or other anti-acne agents.

Examples of oils comprise fats and oils such as olive oil and hydrogenated oils; waxes such as beeswax and lanolin; hydrocarbons such as liquid paraffin, ceresin, and squalane; fatty acids such as stearic acid and oleic acid; alcohols such as cetyl alcohol, stearyl alcohol, lanolin alcohol, and hexadecanol; and esters such as isopropyl myristate, isopropyl palmitate and butyl stearate. As examples of surfactants there may be cited anionic surfactants such as sodium stearate, sodium cetylsulfate, polyoxyethylene laurylether phosphate, sodium N-acyl glutamate; cationic surfactants such as stearyldimethylbenzylammonium chloride and stearyltrimethylammonium chloride; ampholytic surfactants such as alkylaminoethylglycine hydrocloride solutions and lecithin; and nonionic surfactants such as glycerin monostearate, sorbitan monostearate, sucrose fatty acid esters, propylene glycol monostearate, polyoxyethylene oleylether, polyethylene glycol monostearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene coconut fatty acid monoethanolamide, polyoxypropylene glycol (e.g. the materials sold under the trademark "Pluronic"), polyoxyethylene castor oil, and polyoxyethylene lanolin. Examples of humectants include glycerin, 1,3-butylene glycol, and propylene glycol; examples of lower alcohols include ethanol and isopropanol; examples of thickening agents include xanthan gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, polyethylene glycol and sodium carboxymethyl cellulose; examples of antioxidants comprise butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, citric acid and ethoxyquin; examples of chelating agents include disodium edetate and ethanehydroxy diphosphate; examples of buffers comprise citric acid, sodium citrate, boric acid, borax, and disodium hydrogen phosphate; and examples of preservatives are methyl parahydroxybenzoate, ethyl parahydroxybenzoate, dehydroacetic acid, salicylic acid and benzoic acid.

For preparing ointments, creams, toilet waters, skin milks, and the like, typically from 0.01 to 10% in particular from 0.1 to 5% and more in particular from 0.2 to 2.5% of the active ingredient, e.g., of the TGFβ therapeutic, will be incorporated in the compositions. In ointments or creams, the carrier for example consists of 1 to 20%, in particular 5 to 15% of a humectant, 0.1 to 10% in particular from 0.5 to 5% of a thickener and water; or said carrier may consist of 70 to 99%, in particular 20 to 95% of a surfactant, and 0 to 20%, in particular 2.5 to 15% of a fat; or 80 to 99.9% in particular 90 to 99% of a thickener; or 5 to 15% of a surfactant, 2-15% of a humectant, 0 to 80% of an oil, very small

(< 2%) amounts of preservative, coloring agent and/or perfume, and water. In a toilet water, the carrier for example consists of 2 to 10% of a lower alcohol, 0.1 to 10% or in particular 0.5 to 1% of a surfactant, 1 to 20%, in particular 3 to 7% of a humectant, 0 to 5% of a buffer, water and small amounts (< 2%) of preservative, dyestuff and/or perfume. In a skin milk, the carrier typically consists of 10-50% of oil, 1 to 10% of surfactant, 50-80% of water and 0 to 3% of preservative and/or perfume. In the aforementioned preparations, all % symbols refer to weight by weight percentage.

Particular compositions for use in the method of the present invention are those wherein the TGFβ therapeutic is formulated in liposome-containing compositions. Liposomes are artificial vesicles formed by amphiphatic molecules such as polar lipids, for example, phosphatidyl cholines, ethanolamines and serines, sphingomyelins, cardiolipins, plasmalogens, phosphatidic acids and cerebiosides. Liposomes are formed when suitable amphiphathic molecules are allowed to swell in water or aqueous solutions to form liquid crystals usually of multilayer structure comprised of many bilayers separated from each other by aqueous material (also referred to as coarse liposomes). Another type of liposome known to be consisting of a single bilayer encapsulating aqueous material is referred to as a unilamellar vesicle. If water-soluble materials are included in the aqueous phase during the swelling of the lipids they become entrapped in the aqueous layer between the lipid bilayers.

Water-soluble active ingredients such as, for example, various salt forms of a TGFβ polypeptide, are encapsulated in the aqueous spaces between the molecular layers. The lipid soluble active ingredient of TGFβ therapeutic, such as an organic mimetic, is predominantly incorporated into the lipid layers, although polar head groups may protude from the layer into the aqueous space. The encapsulation of these compounds can be achieved by a number of methods. The method most commonly used involves casting a thin film of phospholipid onto the walls of a flask by evaporation from an organic solvent. When this film is dispersed in a suitable aqueous medium, multilamellar liposomes are formed. Upon suitable sonication, the coarse liposomes form smaller similarly closed vesicles.

Water-soluble active ingredients are usually incorporated by dispersing the cast film with an aqueous solution of the compound. The unencapsulated compound is then removed by centrifugation, chromatography, dialysis or other art-known suitable procedures. The lipid-soluble active ingredient is usually incorporated by dissolving it in the organic solvent with the phospholipid prior to casting the film. If the solubility of the material in the lipid

phase is not exceeded or the amount present is not in excess of that which can be bound to the lipid, liposomes prepared by the above method usually contain most of the material bound in the lipid bilayers; separation of the liposomes from unencapsulated material is not required.

5 A particularly convenient method for preparing liposome formulated forms of TGFβ therapeutics is the method described in EP-A-253,619, incorporated herein by reference. In this method, single bilayered liposomes containing encapsulated active ingredients are prepared by dissolving the lipid component in an organic medium, injecting the organic solution of the lipid component under pressure into an aqueous component while 10 simultaneously mixing the organic and aqueous components with a high speed homogenizer or mixing means, whereupon the liposomes are formed spontaneously.

The single bilayered liposomes containing the encapsulated TGFβ therapeutic can be employed directly or they can be employed in a suitable pharmaceutically acceptable carrier for topical administration. The viscosity of the liposomes can be increased by the 15 addition of one or more suitable thickening agents such as, for example xanthan gum, hydroxypropyl cellulose, hydroxypropyl methylcellulose and mixtures thereof. The aqueous component may consist of water alone or it may contain electrolytes, buffered systems and other ingredients, such as, for example, preservatives. Suitable electrolytes which can be employed include metal salts such as alkali metal and alkaline earth metal salts. The 20 preferred metal salts are calcium chloride, sodium chloride and potassium chloride. The concentration of the electrolyte may vary from zero to 260 mM, preferably from 5 mM to 160 mM. The aqueous component is placed in a suitable vessel which can be adapted to effect homogenization by effecting great turbulence during the injection of the organic component. Homogenization of the two components can be accomplished within the vessel, 25 or, alternatively, the aqueous and organic components may be injected separately into a mixing means which is located outside the vessel. In the latter case, the liposomes are formed in the mixing means and then transferred to another vessel for collection purpose.

The organic component consists of a suitable non-toxic, pharmaceutically acceptable solvent such as, for example ethanol, glycerol, propylene glycol and 30 polyethylene glycol, and a suitable phospholipid which is soluble in the solvent. Suitable phospholipids which can be employed include lecithin, phosphatidylcholine, phosphatydylserine, phosphatidylethanol-amine, phosphatidylinositol, lysophosphatidylcholine and phospha-tidyl glycerol, for example. Other lipophilic additives

may be employed in order to selectively modify the characteristics of the liposomes. Examples of such other additives include stearylamine, phosphatidic acid, tocopherol, cholesterol and lanolin extracts.

In addition, other ingredients which can prevent oxidation of the phospholipids may be added to the organic component. Examples of such other ingredients include tocopherol, butylated hydroxyanisole, butylated hydroxytoluene, ascorbyl palmitate and ascorbyl oleate. Preservatives such a benzoic acid, methyl paraben and propyl paraben may also be added.

Apart from the above-described compositions, use may be made of covers, e.g. plasters, bandages, dressings, gauze pads and the like, containing an appropriate amount of a TGFβ therapeutic. In some cases use may be made of plasters, bandages, dressings, gauze pads and the like which have been impregnated with a topical formulation containing the therapeutic formulation.

Exemplification

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Example 1: TGFβ Proteins Stimulate Expansion/Growth of Pancreatic Progenitor Cells

TGFβ proteins are stable, multifunctional polypeptide growth factors. Proteins in this family have effects on growth and differentiation of many cell types. We tested the effects of TGFβ -1, -2, -3, and -5 on ductal explants to determine if these closely related growth factors (70-80% sequence homology) had any effects on growth and/or differentiation of pancreatic progenitor cells.

Methods

Briefly, the main pancreatic duct was dissected from an adult rat or mouse. The duct was cleaned of fat and pancreatic tissue. The duct was sectioned into 200 micron sections and placed on ice. A 12- or 24-well plate was coated with matrigel-reduced growth

factor. The matrigel was allowed to solidify for 15 minutes at room temperature. The ducts were individually placed on top of the matrigel. To allow the ducts to adhere to the matrigel, the plate was incubated at room temperature for 30 minutes. After 30 minutes, DMEM (low glucose) was added to each well. TGFβ protein, at appropriate concentrations, was added to the culture. The culture was incubated at 37 degrees C with 5% CO_2. Every 3-4 days, spent media was removed and fresh media and TGFβ was added.

To assess PDX-1 gene expression, transgenic mice with a PDX-1/LacZ construct were generated and used as a source of duct explants as described above.

Results

TGFβ -2 (200pg/ml), TGFβ -3 (30 pg/ml) and TGFβ -5 (30 pg/ml) all caused substantial growth of rat ductal explants (see figure 1). TGFb-1 and DMEM alone both had little or no effect. TGFβ -5 was the most potent activator of growth. Similar results were seen with mouse ductal explants.

To verify that expansion of ductal explant was due to growth of cells of pancreatic lineage, the effect of TGFβ proteins on PDX-1 gene expression was determined. Ductal explants were obtained from PDX-1/LacZ mice as described above and cultured in the presence of TGFβ proteins. Again, TGFβ -5 caused the greatest increase in PDX-1 positive nuclei. TGFβ -2 and TGFβ -3 also caused an increase PDX-1-expressing cells.

These experiments demonstrate that TGFβ -2, -3 and -5 proteins can stimulate expansion of pancreatic ductal tissue in vitro.

Example 2: TGFβ Proteins Stimulate Pancreatic Expansion in vivo

In vitro culture of adult ductal explants with the multifunctional polypeptide growth factor family of TGFβs, indicated that TGFβ -2, TGFβ -3 and TGFβ -5 but not TGFβ -1, stimulate proliferation and activation of PDX-1 in the duct epithelial cells in the adult explant. To demonstrate whether these factors would also cause proliferation and activation of PDX-1 in the duct epithelial cells of the common pancreatic duct, we tested the in vivo effect of TGFβ -1, TGFβ -2, TGFβ -3 and TGFβ -5 in adult mice.

Protocol

8 week old female C57Bl/6 mice were injected i.p. with vehicle (PBS or mouse serum albumin) or with 0.54ng/gm of TGFβ -1, TGFβ -2, TGFβ -3 and TGFβ -5 for 3 consecutive days. The injections were given once per day in a total volume of 200ul in PBS and a minimum of 3-5 mice per group were analyzed (Fig 1). The mice were analyzed in two different ways. They were either pulsed with BrdU for 6 hours on the third day of treatment with TGFβs and then sacrificed or they were sacrificed on the 5th day. In both analyses, pancreata and the common pancreatic duct from the mice were collected, fixed, paraffin sectioned and stained for PDX-1, insulin, glucagon and BrdU (see page 43 of patent for method). The study design is depicted in figure X.

To determine the effects of TGFβ treatment on the insulin/glucagons homeostatic system, plasma glucose, plasma insulin and body weights were recorded before and after treatment with vehicle or with TGFβ proteins on the 5th day of sacrifice (Fig 2, 3 & 4).

Results and Methods

The body weights were measured before (day 0) and after 1 month's treatment with vehicle (PBS or mouse serum albumin) or TGFβ protein. Body weight was not significantly affected by TGFβ protein treatment.

Between 3-5 age matched animals per group were fasted overnight for approximately 16 hours before (Day -3) and after (Day 4). Mice were bled retroorbitally on Day 5 and fasting blood glucose concentrations were measured with a portable glucose meter (Accu-check: Boehringer Mannheim Biochemicals).

As can be observed, after 5 days of treatment the mice in all the groups were normoglycemic.

Between 3-5 age matched animals per group were fasted overnight for approximately 16 hours before (Day -3) and after (Day 4). Mice were bled retroorbitally on Day 5 into heparinized capillary tubes and plasma separated by spinning in a centrifuge for 10min at 4oC.

After 16hrs of fasting, plasma insulin levels were moderately higher for TGFβ -2 and TGFβ -3 than the vehicle littermates suggesting that TGFβ -2 and TGFβ -3 treated mice were leaning towards being mildly hyperinsulinemic.

Proliferation in the common pancreatic duct was determined by 5-bromo-2'-deoxyuridine (BrdU) incorporation. Untreated, TGFβ -1 and TGFβ -5 mice were given a single dose of BrdU (100Ug/gm body weight i.p.) dissolved in 7mM NaOH in normal saline. After 6 hours of incorporation, mice were sacrificed and pancreata and the common bile duct were fixed in 4% PFA overnight. The tissues were then washed in PBS, embedded, sectioned, deparaffinized and stained with BrdU antibody (red Cy3 staining) and Insulin (green FITC staining) antibody .

We observed BrdU + cells in the ductal epithelial cells indicating proliferation in the TGFβ -5 treated mice but not in untreated or TGFβ -1 treated mice. Some of the BrdU+ cells were also insulin positive in the TGFb5 animals suggesting that TGFβ -5 stimulates proliferation and differentiation of the duct epithelial cells.

Total Islet area are increased in the TGFβ -3 and TGFβ -5 treated mice. Pancreatic sections from vehicle and TGFβ -b treated mice sacrificed on day 5 were co-stained for insulin and glucagon.

The total islet area per unit of exocrine pancreas volume was observed to be marked increased in TGFβ -2 and TGFβ -3 treated mice. Immunostaining with insulin (green FITC staining) and glucagon (red Cy3 staining) indicated that that the morphology and architecture of the islets had been conserved in these animals.

In order to determine whether the islet area had increased, the area of islets in the untreated, TGFβ -1 and TGFβ -5 treated mice were quantitated using a Nikon 800 microscope and analyzed using the Optimus software package. 2 sections from each animal (2 animals from a different study group were analysed) and 10 islets per section were analysed to quantitate the islet area.

The quantitation showed that the islet area was not significantly changed between untreated and TGFβ -1 treated animals. In contrast, the islet area of TGFβ -5 treated animals was found to be increased by 7 fold compared to the control.

Example 3: TGFβ-4

Purpose: To test whether TGFB-4 (EBAF) has any proliferative/expansion effects on ductal explants in the adult rat assay. Note that TGFB-2, TFGB-3, and TGFB-5, but not TGFB-1 had proliferative effects on growth and expansion potential with respect to PDX-1, demonstrated in PDX-1/LacZ duct explants.

Method

1. Dissect out the pancreatic ducts from 6 week old female adult rats.
2. Transfer to cold Iscoves Buffer (P/.S) on ice.
3. Transfer one duct to cold Iscoves Medium (P/S) and clean off pancreatic and fat tissue. Clean ducts one at a time in fresh medium.
4. Transfer clean duct to fresh Iscoves Medium.
5. Let ducts sit on ice for 10 minutes.
6. Place two ducts at a time on the sectioning platform and section.
7. Carefully transfer sections to fresh cold Iscoves in a 35mm dish.
8. Let sections sit on ice for 20 minutes.
9. Add DMEM (p/s/g) + TGFB-4 at 10% and 20% CM
10. Add 10 sections of duct per well of 12-well plate coated with Matrigel-Reduced growth factor.
11. Incubate at 37 C with 5% CO_2
12. Fed every 3-4 days with fresh medium +/-TGFB-4.
13. Check Cultures regularly and teminate after 2 weeks in culture.

Results: No growth with 10% or 20% TGFB-4 conditioned medium.

All of the above-cited references and publications are hereby incorporated by reference.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. A method of treating a subject with a disorder resulting from insufficient insulin production comprising contacting said subject with a TGFβ therapeutic.

2. A method of claim 1 wherein said TGFβ therapeutic is a polypeptide comprising a TGFβ protein.

3. A method of claim 2 wherein said TGFβ protein is a member of the TGFβ protein subfamily.

4. A method of claim 2 wherein said TGFβ protein is at least 60% identical to one or more of the following: SEQ. ID. No: 2, SEQ. ID. No: 3, or SEQ. ID. No: 5.

5. A method of claim 2 wherein said TGFβ protein is at least 70% identical to one or more of the following: SEQ. ID. No: 7, SEQ. ID. No: 8, or SEQ. ID. No: 10.

6. A method of claim 2 wherein said TGFβ protein comprises one or more of the following: SEQ. ID. No: 11, SEQ. ID. No: 12, or SEQ. ID. No: 13.

7. A method of claim 1 wherein said TGFβ therapeutic is a TGFβ agonist.

8. A method of claim 1 wherein said disorder is diabetes.

9. A method of claim 1 wherein said disorder is Type I diabetes.

10. A pharmaceutical composition comprising a polypeptide at least 50% identical to SEQ. ID. No. 5 and a pharmaceutically acceptable carrier.

11. A pharmaceutical composition comprising a polypeptide at least 76% identical to SEQ. ID. No. 10 and a pharmaceutically acceptable carrier.

12. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a polypeptide comprising one of SEQ. ID. Nos. 11-13.

13. A method of inducing outgrowth of pancreatic cells comprising contacting said cells with a TGFβ therapeutic.

14. A method of claim 13 wherein said TGFβ therapeutic is a polypeptide comprising a TGFβ protein.

15. A method of claim 14 wherein said TGFβ protein is a member of the TGFβ protein subfamily.

16. A method of claim 14 wherein said TGFβ protein is at least 60% identical to one or more of the following: SEQ. ID. No: 2, SEQ. ID. No: 3, or SEQ. ID. No: 5.

17. A method of claim 14 wherein said TGFβ protein is at least 70% identical to one or more of the following: SEQ. ID. No: 7, SEQ. ID. No: 8, or SEQ. ID. No: 10.

18. A method of claim 14 wherein said TGFβ protein comprises one or more of the following: SEQ. ID. No: 11, SEQ. ID. No: 12, or SEQ. ID. No: 13.

19. A method of claim 1 wherein said TGFβ therapeutic comprises a TGFβ agonist.

20. A method of claim 13 wherein said cells are associated with pancreatic duct tissue.

21. A method of claim 13 wherein said cells are within a subject.

Human TGFβ-1 Amino Acid Sequence (SEQ. ID. No.1)

```
  1 mppsglrlll lllpllwllv ltpgrpaagl stcktidmel vkrkrieair gqilsklrla
 61 sppsqgevpp gplpeavlal ynstrdrvag esaepepepe adyyakevtr vlmvethnei
121 ydkfkqsths iymffntsel reavpepvll sraelrllrl klkveqhvel yqkysnnswr
181 ylsnrllaps dspewlsfdv tgvvrqwlsr ggeiegfrls ahcscdsrdn tlqvdingft
241 tgrrgdlati hgmnrpflll matpleraqh lqssrhrral dtnycfsste knccvrqlyi
301 dfrkdlgwkw ihepkgyhan fclgpcpyiw sldtqyskvl alynqhnpga saapccvpqa
361 leplpivyyv grkpkveqls nmivrsckcs
```

Figure 1

Human TGFβ-2 Amino Acid Sequence (SEQ. ID. No.2)

```
  1 mhycvlsafl ilhlvtvals lstcstldmd qfmrkrieai rgqilsklkl tsppedypep
 61 eevppevisi ynstrdllqe kasrraaace rersdeeyya kevykidmpp ffpseaippt
121 fyrpyfrivr fdvsamekna snlvkaefrv frlqnpkarv peqrielyqi lkskdltspt
181 qryidskvvk traegewlsf dvtdavhewl hhkdrnlgfk islhcpcctf vpsnnyiipn
241 kseelearfa gidgtstyts gdqktikstr kknsgktphl llmllpsyrl esqqtnrrkk
301 raldaaycfr nvqdncclrp lyidfkrdlg wkwihepkgy nanfcagacp ylwssdtqhs
361 rvlslyntin peasaspccv sqdlepltil yyigktpkie qlsnmivksc kcs
```

Figure 2

Human TGFβ-3 Amino Acid Sequence (SEQ. ID. No.3)

```
  1 mkmhlqralv vlallnfatv slslstcttl dfghikkkrv eairgqilsk lrltsppept
 61 vmthvpyqvl alynstrell eemhgereeg ctqentesey yakeihkfdm iqglaehnel
121 avcpkgitsk vfrfnvssve knrtnlfrae frvlrvpnps skrneqriel fqilrpdehi
181 akqryiggkn lptrgtaewl sfdvtdtvre wllrresnlg leisihcpch tfqpngdile
241 nihevmeikf kgvdneddhg rgdlgrlkkq kdhhnphlil mmipphrldn pgqggqrkkr
301 aldtnycfrn leenccvrpl yidfrqdlgw kwvhepkgyy anfcsgpcpy lrsadtthst
361 vlglyntlnp easaspccvp qdlepltily yvgrtpkveq lsnmvvksck cs
```

Figure 3

Human TGFβ-4 Amino Acid Sequence (SEQ. ID. No.4)

```
  1 mwplwlcwal wvlplagpga alteeqllas llrqlqlsev pvldradmek lvipahvraq
 61 yvvllrrdgd rsrgkrfsqs frevagrfla seasthllvf gmeqrlppns elvqavlrlf
121 qepvpqgalh rhgrlspaap karvtvewlv rddgsnrtsl idsrlvsvhe sgwkafdvte
181 avnfwqqlsr ppepllvqvs vqrehlgpla sgahklvrfa sqgapaglge pqlelhtldl
241 rdygaqgdcd peapmtegtr ccrqemyidl qgmkwaknwv leppgflaye cvgtcqqppe
301 alafnwpflg prqciaseta slpmivsike ggrtrpqvvs lpnmrvqkcs casdgalvpr
361 rlqhrpwcih
```

Figure 4

Xenopus laevis TGFβ-5 Amino Acid Sequence (SEQ. ID. No.5)

```
  1 mevlwmllvl lvlhlsslam slstckavdm eevrkrriea irgqilsklk ldktpdvdse
 61 kmtvpseaif lynstlevir ekatreeehv ghdqniqdyy akqvyrfesi teledhefkf
121 kfnashvren vgmnsllhha elrmykkqtd knmdqrmelf wkyqengtth sryleskyit
181 pvtddewmsf dvtktvnewl kraeeneqfg lqpackcptp qakdidiegf palrgdlasl
241 sskentkpyl mitsmpaeri dtvtssrkkr gvgqeycfgn ngpnccvkpl yinfrkdlgw
301 kwihepkgye anyclgncpy iwsmdtqysk vlslynqnnp gasispccvp dvleplpiiy
361 yvgrtakveq lsnmvvrscn cs
```

Figure 5

Human TGFβ-1 C-terminal 112 Amino Acid Sequence (SEQ. ID. No.6)

al dtnycfsste knccvrqlyi dfrkdlgwkw ihepkgyhan fclgpcpyiw sldtqyskvl

alynqhnpga saapccvpqa leplpivyyv grkpkveqls nmivrsckcs

Figure 6

Human TGFβ-2 112 C-Terminal Amino Acid Sequence (SEQ. ID. No.7)

aldaaycfr nvqdncclrp lyidfkrdlg wkwihepkgy nanfcagacp ylwssdtqhs rvlslyntin

peasaspccv sqdlepltil yyigktpkie qlsnmivksc kcs

Figure 7

Human TGFβ-3 C-terminal 112 Amino Acid Sequence (SEQ. ID. No.8)

aldtnycfrn leenccvrpl yidfrqdlgw kwvhepkgyy anfcsgpcpy lrsadtthst vlglyntlnp

easaspccvp qdlepltily yvgrtpkveq lsnmvvksck cs

Figure 8

Human TGFβ-4 C-terminal Amino Acid Sequence (SEQ. ID. No.9)

gtr ccrqemyidl qgmkwaknwv leppgflaye cvgtcqqppe alafnwpflg prqciaseta

slpmivsike ggrtrpqvvs lpnmrvqkcs casdgalvpr rlqhrpwcih

Figure 9

Xenopus laevis TGFβ-5 C-terminal 112 Amino Acid Sequence (SEQ. ID. No.10)

gvgqeycfgn ngpnccvkpl yinfrkdlgw kwihepkgye anyclgncpy iwsmdtqysk vlslynqnnp

gasispccvp dvleplpiiy yvgrtakveq lsnmvvrscn cs

Figure 10

TGFβ-5 Motifs

Motif No. 1 (SEQ. ID. No. 11):

(G/P) (V/I) (G/A/P) (Q/N) (E/D) XCX (G/A/P) X (N/Q) (G/A/S/T/P) (P/G) $XCCX_6$ (N/Q) X_{16} (E/D/Q) X_3CX_2 (N/Q/D/E/K/R) CX_{19} (N/Q/D/E/K/R) X_5 (I/L/V) X_2CCX_2 (D/E) (I/L/V) X_{13} (A/G) $X_{12}C$ (N/Q) CX

Motif No. 2 (SEQ. ID. No. 12):

$X_6CX_7CCX_{27}CX_2$ (N/Q/D/E/K/R) CX_{19} (N/Q/D/E/K/R) $X_8CCX_{30}CXCX$

Motif No. 3 (SEQ. ID. No.13):

X_6CX_1 (G/A/P) X_2 (G/A/S/T/P) $X_2CCX_{27}CX_3CX_{28}CCX_{30}CXCX$

"X" denotes any of the standard 20 amino acids. Brackets delineate positions where two or more amino acids can be used. For example "(G/P)" indicates that either a glycine or proline may be used at that position.

Figure 11

TGFβ Protein Alignment

```
             1            15 16            30 31            45 46            60 61            75 76            90
1 tgfb2h     ---------MHYCVL SAFLILHLVTVALSL STCSTLDMDQFMRKR IEAIRGQILSKLKLT SPPEDYPEPEEVPPE VISIYNSTRDLLQEK   81
2 tgfb3h     ------MKMHLQRAL VVLALLNFATVSLSL STCTTLDFGHIKKKR VEAIRGQILSKLRLT SPPE-PTVMTHVPYQ VLALYNSTRELLEEM   83
3 tgfb1h     MPPSGLRLLLLLLPL LWLLVLTPGRPAAGL STCKTIDMELVKRKR IEAIRGQILSKLRLA SPPS---QGEVPPGP LPEAVLALYNSTRDR   87
4 tgfb5f     --------MEVLWML LVLLVLHLSSLAMSL STCKAVDMEEVRKRR IEAIRGQILSKLKLD KTP----DVDSEKMT VPSEAIFLYNSTLEV   78

             91          105 106          120 121          135 136          150 151          165 166          180
1 tgfb2h     ASRRAAACERERSDE EYYAKEVYKIDMPP- FFPSEAIPPTFYRPY FRIVRFDVSAMEKNA SNLVKAEFRVFRLQN PKARVPEQRIELYQI  170
2 tgfb3h     HGEREEGCTQENTES EYYAKEIHKFDMIQG LAEHNELAVCPKGIT SKVFRFNVSSVEKNR TNLFRAEFRVLRVPN PSSKRNEQRIELFQI  173
3 tgfb1h     VAGESAEPEPEPEAD YYAKEVTRVLMVETH NEIYDKFKQSTHSIY MFFNTSELREAVPEP VLLSRAELRLLR--L KLKVEQHVELYQKYS  175
4 tgfb5f     IR----EKATREEEH VGHDQNIQDYYAKQV YRFESITELEDHEFK FKFNASHVRENVGMN SLLHHAELRMYKKQT DKNMDQRMELFWKYQ  164

             181          195 196          210 211          225 226          240 241          255 256          270
1 tgfb2h     LKSKDLTSPTQRYID SKVVKTRAEGEWLSF DVTDAVHEWLHHKDR NLGFKISLHCPCCTF VPSNNYIIPNKSEEL EARFAGIDGTSTYTS  260
2 tgfb3h     LRP-DEHIAKQRYIG GKNLPTRGTAEWLSF DVTDTVREWLLRRES NLGLEISIHCPCHTF QP-NGDILENIHEVM EIKFKGVDNEDDHGR  261
3 tgfb1h     NN-------SWRYLS NRLLAPSDSPEWLSF DVTGVVRQWLSRGGE IEGFRLSAHCSCDSR DNTLQVDINGFTTGR RGDLATIHGMN----  254
4 tgfb5f     ENG----TTHSRYLE SKYITPVTDDEWMSF DVTKTVNEWLKRAEE NEQFGLQPACKCPTP -QAKDIDIEGFPALR -GDLASLSSKENT--  246

             271          285 286          300 301          315 316          330 331          345 346          360
1 tgfb2h     GDQKTIKSTRKKNSG KTPHLLLMLLPSYRL ESQ-QTNRRKKRALD AAYCFRNVQDNCCLR PLYIDFKRDLGWKWI HEPKGYNANFCAGAC  349
2 tgfb3h     GDLGRLKKQKDHHN- --PHLILMMIPPHRL DNPGQGGQRKKRALD TNYCFRNLEENCCVR PLYIDFRQDLGWKWV HEPKGYYANFCSGPC  348
3 tgfb1h     --------------- -RPFLLLMATPLERA QHL--QSSRHRRALD TNYCFSSTEKNCCVR QLYIDFRKDLGWKWI HEPKGYHANFCLGPC  326
4 tgfb5f     --------------- -KPYLMITSMPAERI DTV--TSSRKKRGVG QEYCFGNNGPNCCVK PLYINFRKDLGWKWI HEPKGYEANYCLGNC  318

             361          375 376          390 391          405 406          420 421          435
1 tgfb2h     PYLWSSDTQHSRVLS LYNTINPEASASPCC VSQDLEPLTILYYIG KTPKIEQLSNMIVKS CKCS       413
2 tgfb3h     PYLRSADTTHSTVLG LYNTLNPEASASPCC VPQDLEPLTILYYVG RTPKVEQLSNMVVKS CKCS       412
3 tgfb1h     PYIWSLDTQYSKVLA LYNQHNPGASAAPCC VPQALEPLPIVYYVG RKPKVEQLSNMIVRS CKCS       390
4 tgfb5f     PYIWSMDTQYSKVLS LYNQNNPGASISPCC VPDVLEPLPIIYYVG RTAKVEQLSNMVVRS CNCS       382
```

The amino acid sequences shown are full pro-proteins. Cleavage releases the C-terminal 112 amino acids that become a mature TGFβ polypeptide. The first amino acid of the mature polypeptide is in large, bold type. Amino acids unique to TGFβ-5 are underlined and italicized.

Figure 12

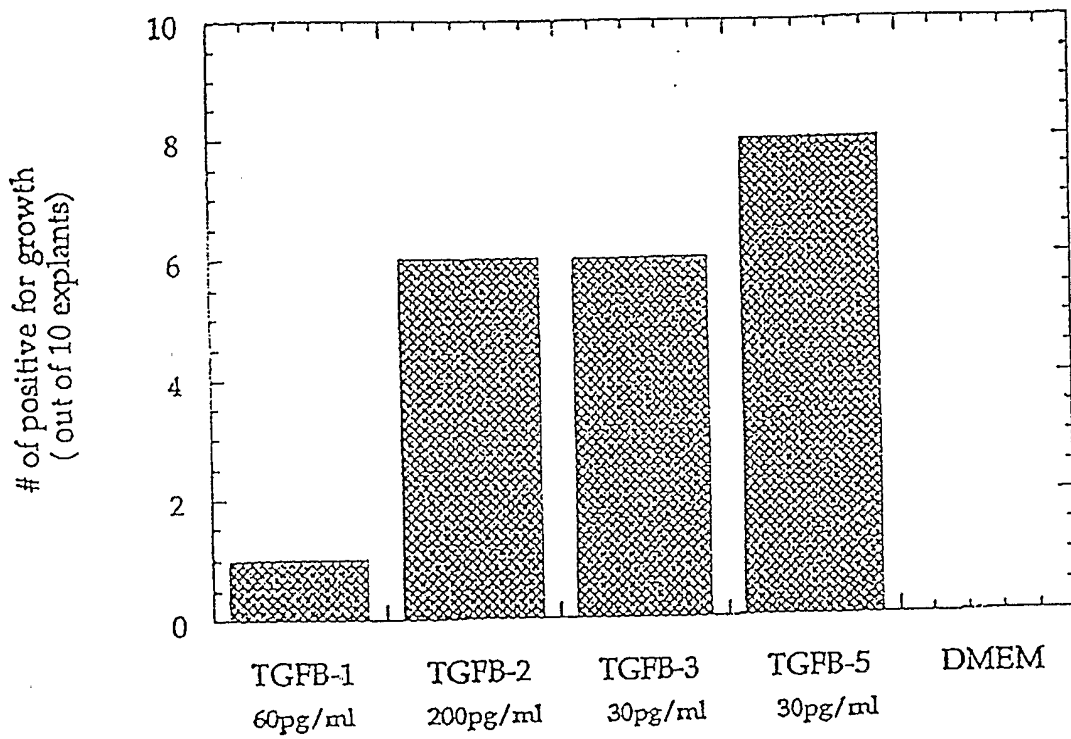

FIGURE 13

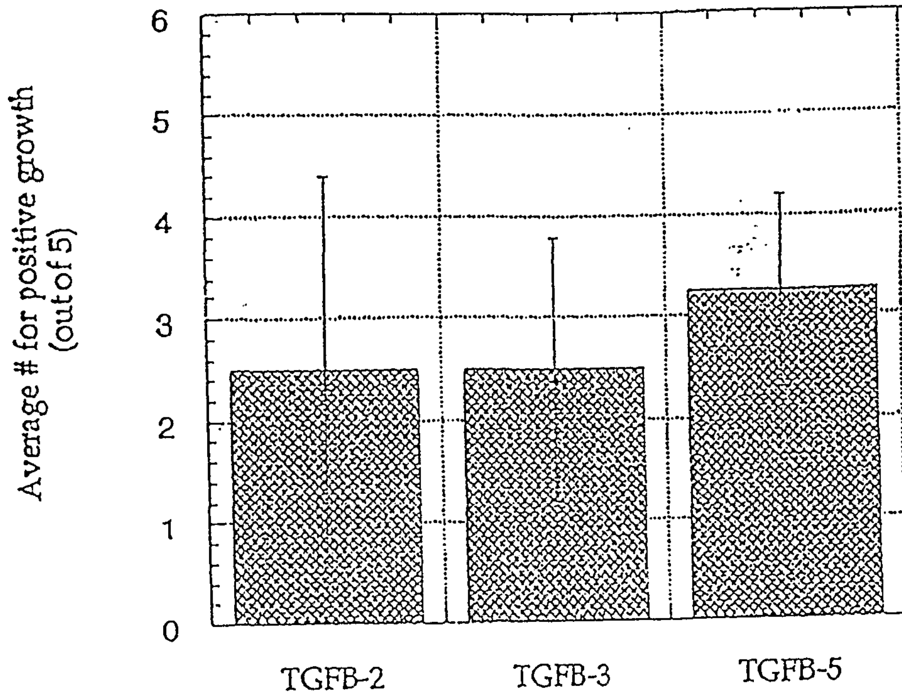

FIGURE 14

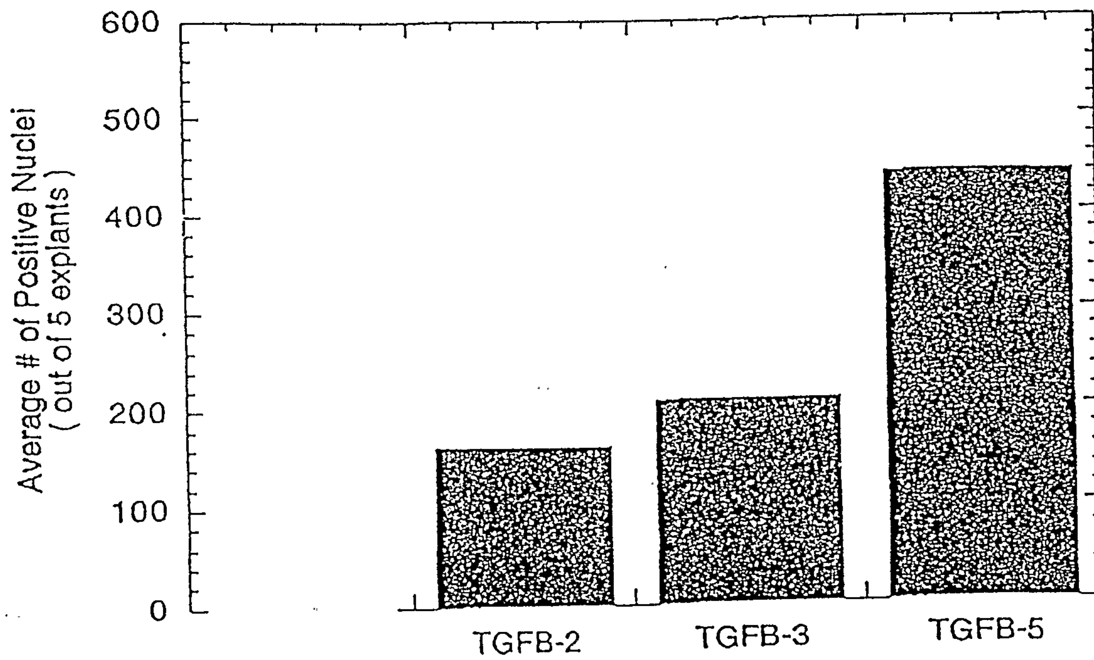

FIGURE 15

LacZ Staining of PDX-1 Duct Explants Treated with TGFB-3 and TGFB-5

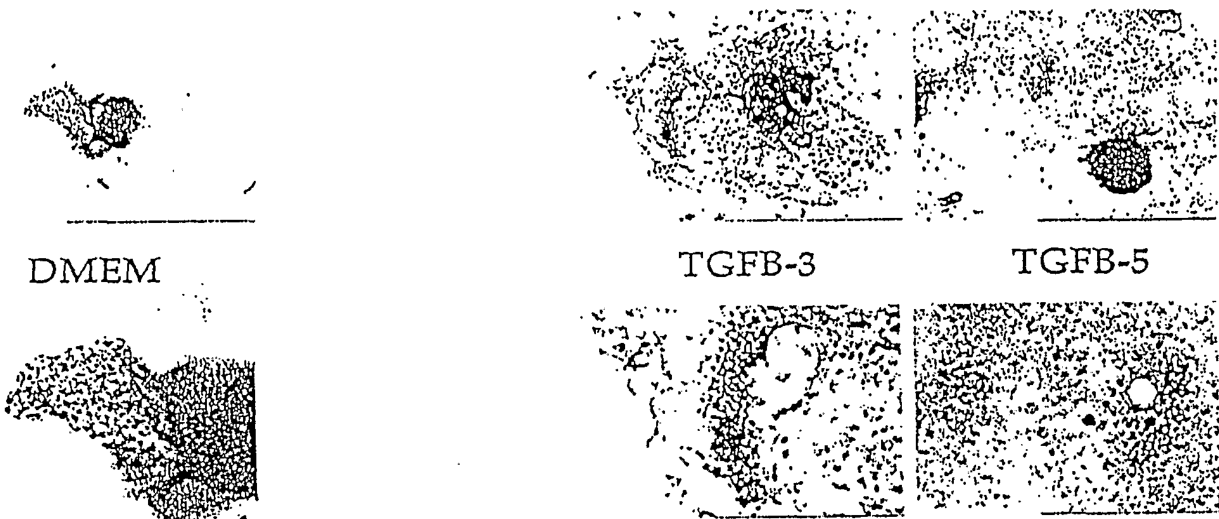

FIGURE 16

FIGURE 17

TGFB-1 ,2, 3, & 5 Stimulates Outgrowth From the Adult Ductal Explant

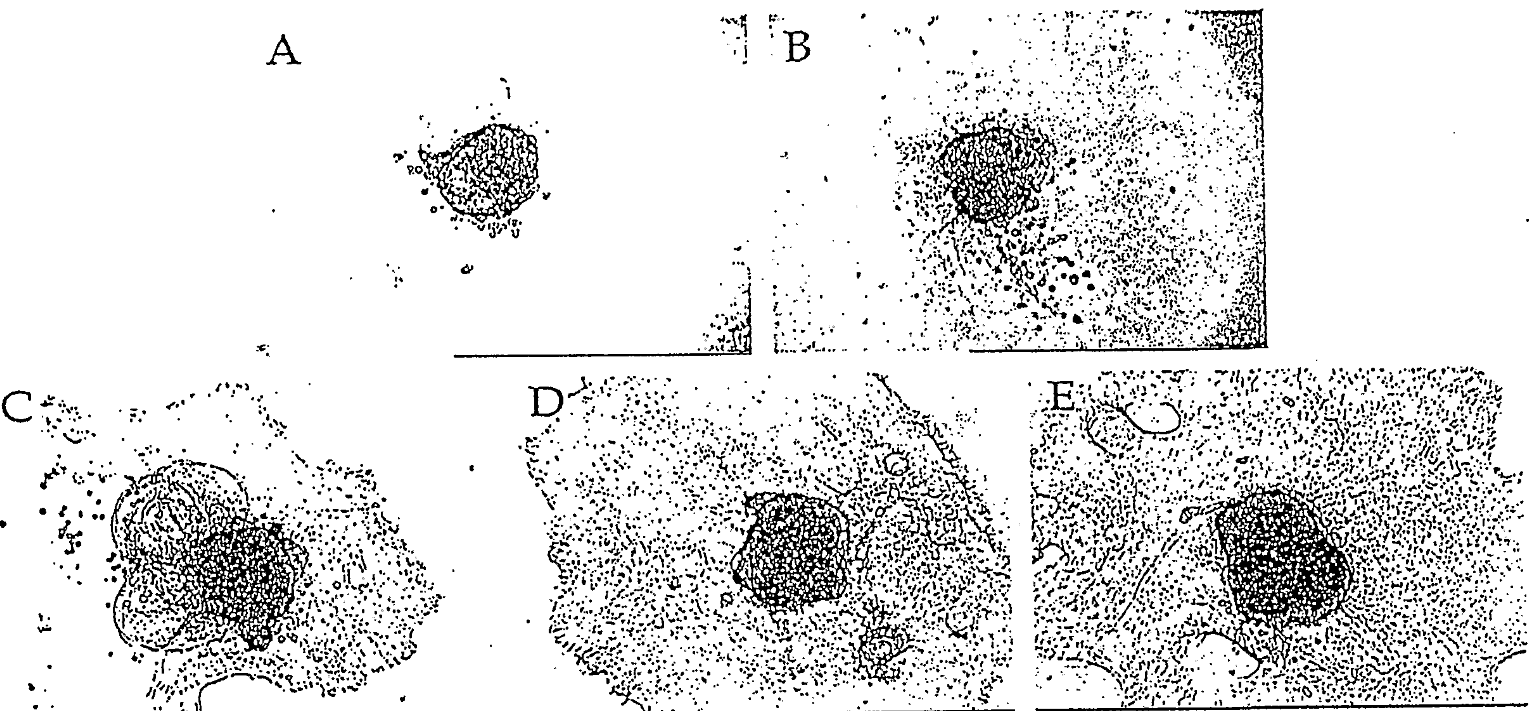

Figure 2a. Adult mouse explants were cultured with either TGFB-1, 2, 3, or 5 for two weeks. Five duct explants were plated per well in a 12-well plate. After two weeks the explants were scored based on outgrowth from original explant. These results have been verified by three other experiments.
A. DMEM treated. B. TGFB-1 at 200pg/ml. C. TGFB-2 at 60pg/ml. D. TGFB-3 at 30pg/ml. E. TGFB-5 at 30pg/ml

Protocol for *in vivo* effects of TGF βs in Mice

Day 0
Day 1
Day 2
Day 3
Day 4
Day 5

8 week old female mice were injected i.p. with 0.54ng/gm of vehicle or TGFβs on 3 consecutive days.

Mice were pulsed for 6 hours with BrdU and then sacrificed

Mice were sacrificed and analyzed

Figure 18

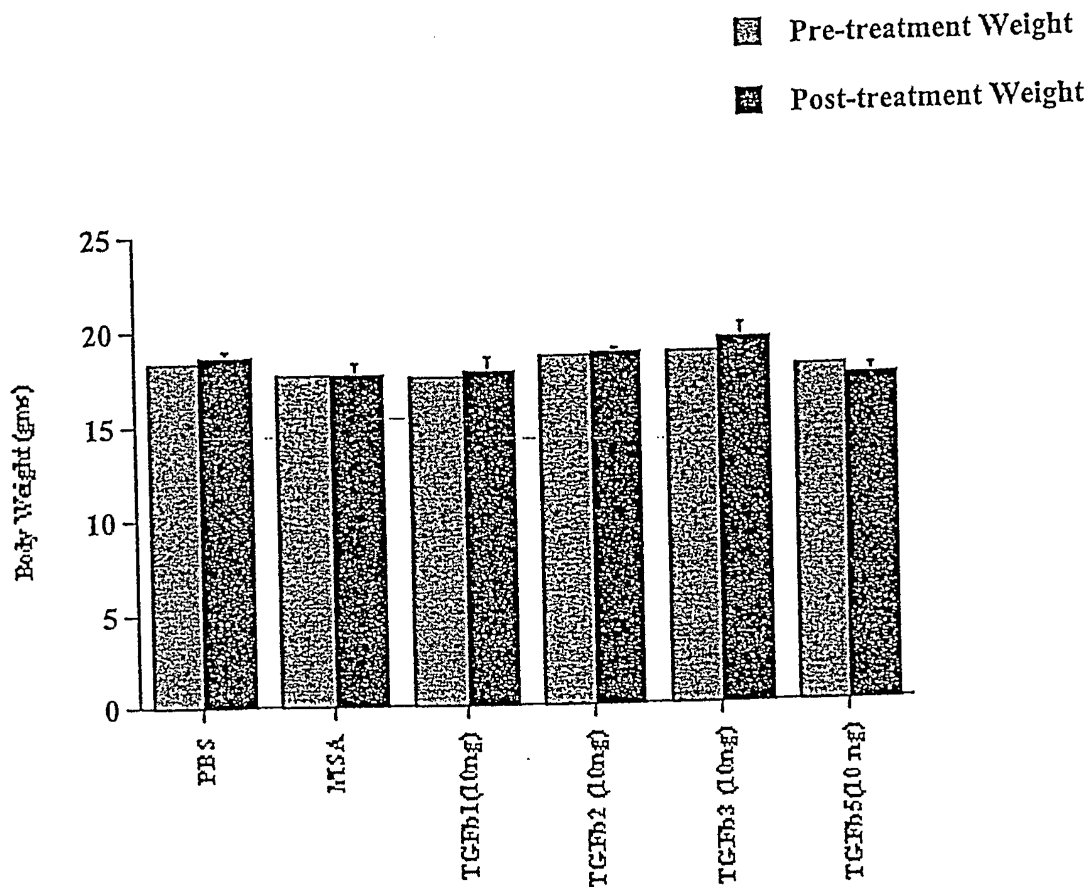

FIGURE 19

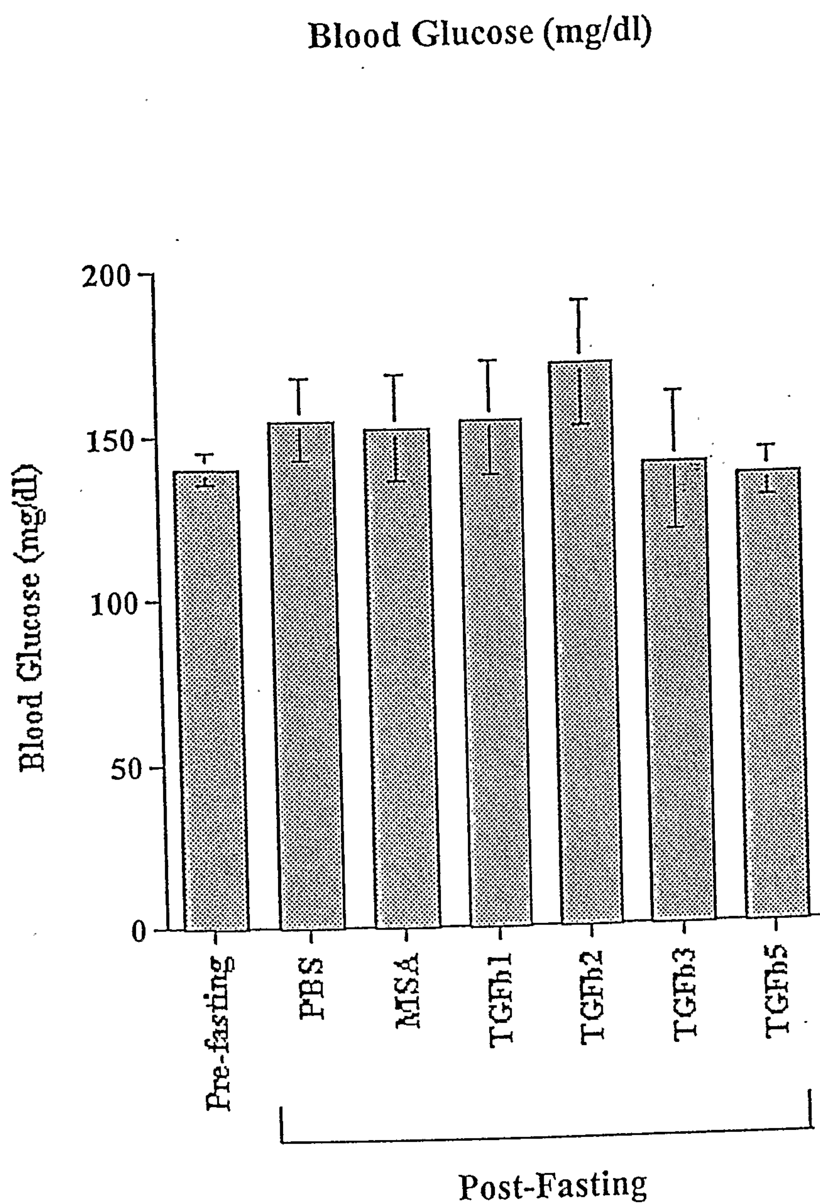

FIGURE 20

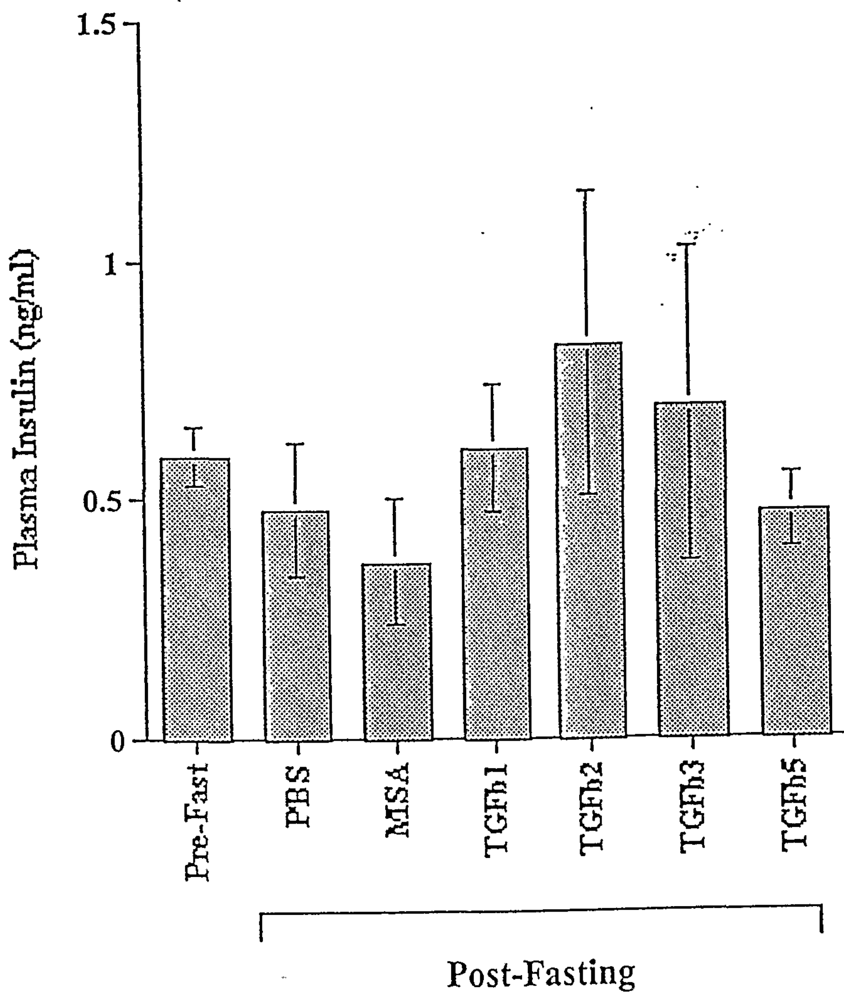

FIGURE 21

6 Hour Systemic Effects of TGFβ-5
on Adult Mouse Common Pancreatic Duct

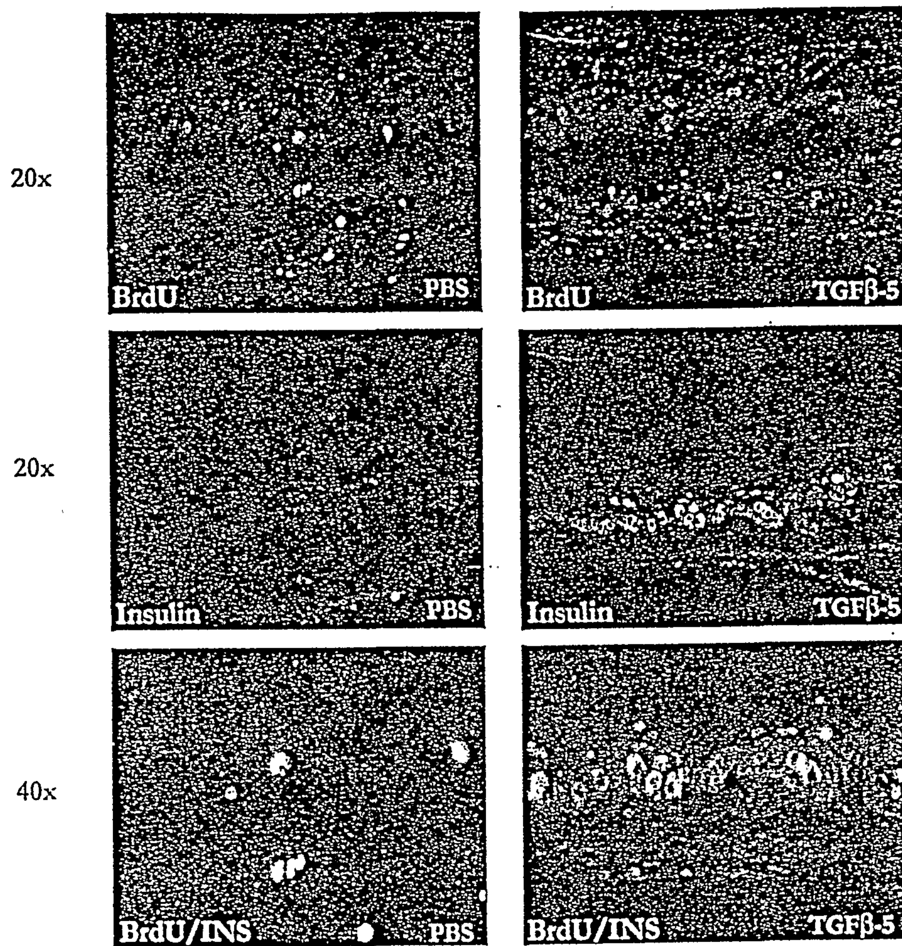

FIGURE 22

Systemic Effects of TGFβs on Adult Mouse Pancreas

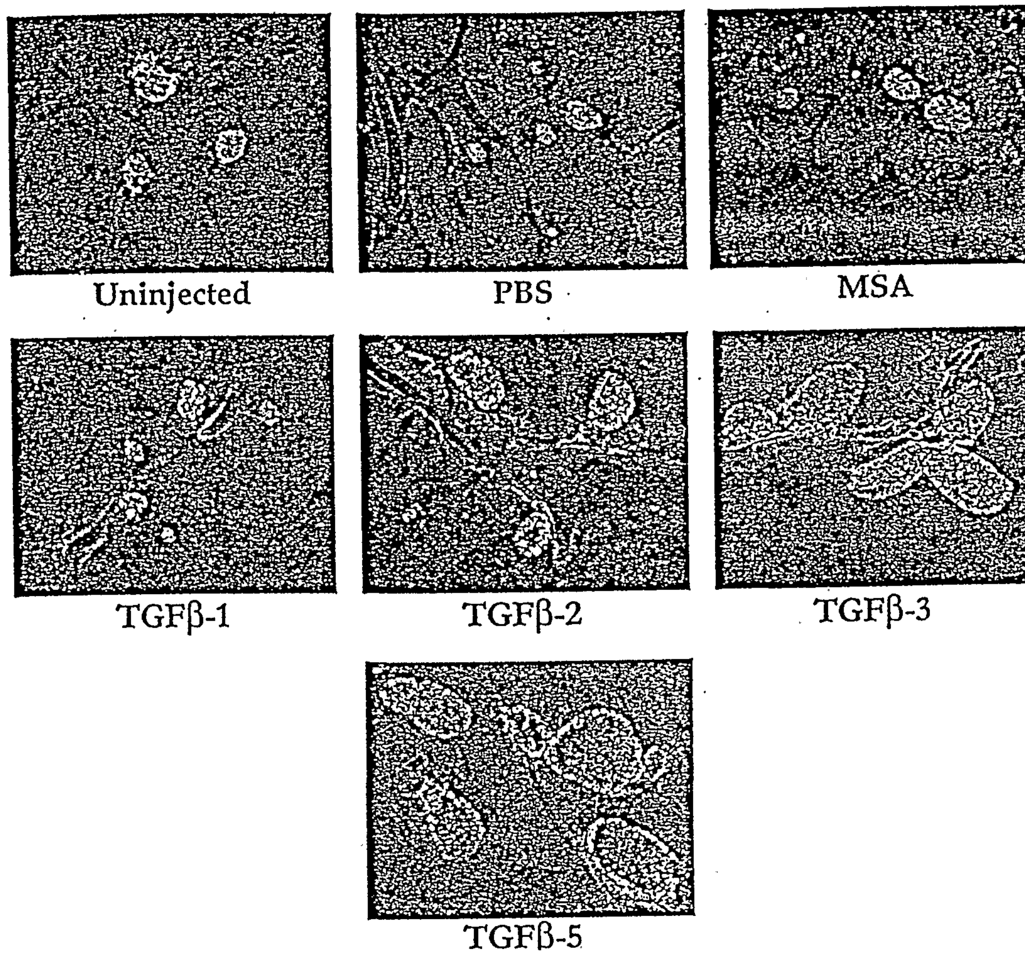

FIGURE 23

Systemic Effects of TGF βs on Islet Cell Area

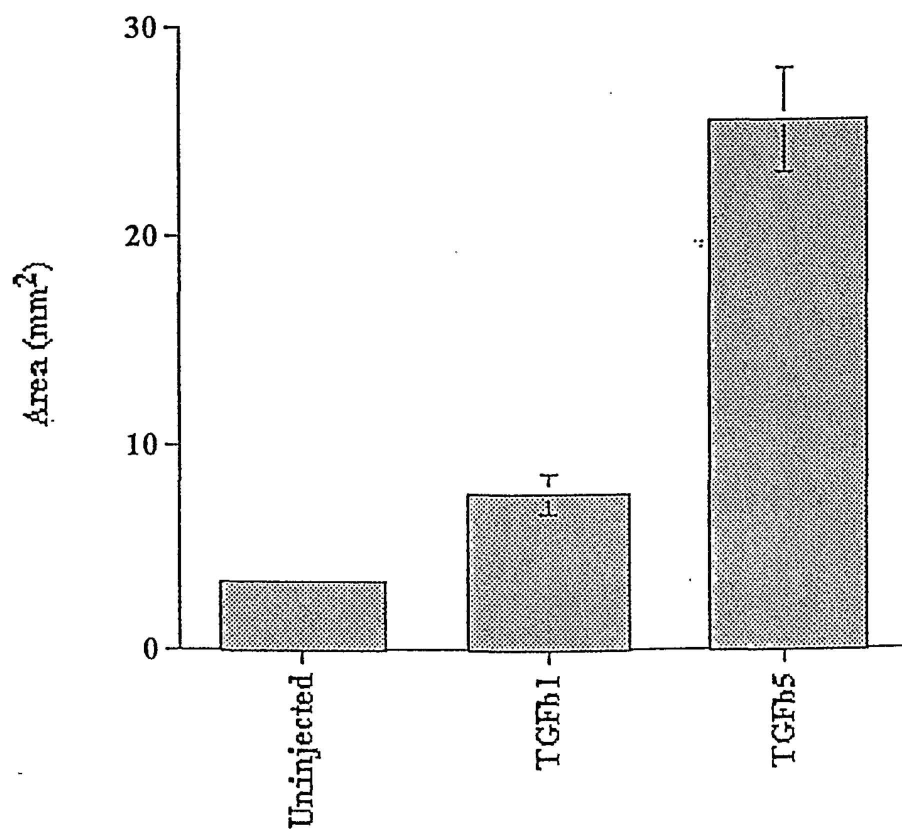

FIGURE 24