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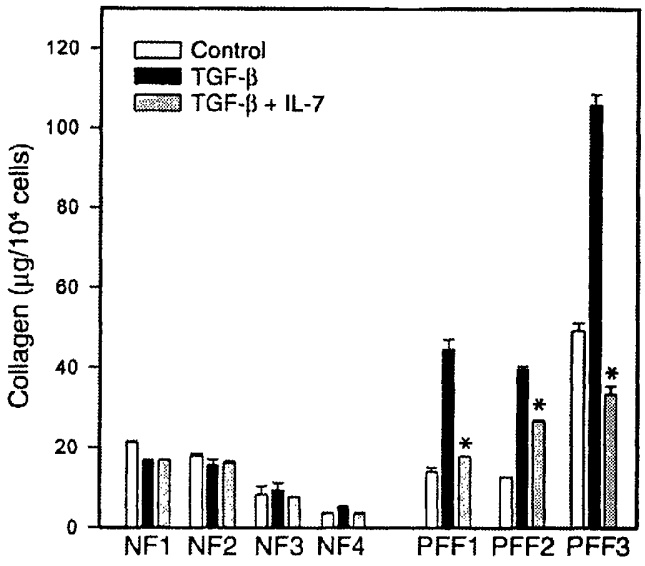

(57) Abstract: The invention is based on the disclosure provided herein on the finding that the cytokine interleukin-7 (IL-7) limits the development of pulmonary fibrosis in clinically relevant models of idiopathic pulmonary fibrosis (IPF). As illustrated below, the disclosure provides additional information relating to the specific cellular mechanisms involved in this pathology and provides models for the assessment of different therapeutic modalities employing IL-7 as well as the assessment of new therapeutic agents and methods for the treatment of this syndrome.

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METHODS OF USING INTERLEUKIN-7 TO MODULATE PHYSIOLOGICAL PROCESSES IN MAMMALIAN PULMONARY FIBROBLASTS

Statement of Government Support

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Related Applications

This application claims priority under Section 119(e) from U.S. Provisional Application Serial No. 60/285,933 filed April 23, 2001, and from U.S. Provisional Application Serial No. 60/286,257 filed April 24, 2001, the contents of each of which are incorporated herein by reference.

Field of the Invention

The present invention relates to methods of using interleukin-7 to modulate physiological processes in mammalian pulmonary fibroblasts including those associated with pathological conditions such as idiopathic pulmonary fibrosis.

Background of the Invention

Respiratory tract disorders are a widespread problem in the United States and throughout the world. Respiratory tract disorders fall into a number of major categories, including inflammatory conditions, infections, cancer, trauma, embolism, and inherited diseases. Lung damage may also be due to physical trauma and exposure to toxins. Inflammatory conditions of the respiratory tract include asthma, chronic obstructive pulmonary disease, sarcoidosis, and idiopathic pulmonary fibrosis (IPF).

Idiopathic pulmonary fibrosis is a devastating disease with less than a 50% five-year survival. While steroids and other immunosuppressive agents serve as the standard

treatment for IPF, these agents have proved inadequate (see e.g. Lynch et al., 1997 *Am. J. Respir. Crit. Care Med.* 155:395–420). In most instances immunosuppressive agents do little to affect the course of the disease and have serious adverse side effects. Thus, novel therapeutic strategies are clearly needed.

The cytokine TGF-β plays a pivotal role in tissue fibrosis including pulmonary fibrosis. Within the normal lung, TGF-β1 is the most abundant isoform and is mostly expressed in interstitial fibroblasts and bronchiolar epithelial cells. Within lungs developing pulmonary fibrotic responses, TGF-β1 is found to be overexpressed in a broad range of cells including fibroblasts, macrophages, and epithelial and endothelial cells. The pathogenesis of pulmonary fibrosis includes the deterioration of the normal homeostatic mechanisms regulating the equilibrium between the synthesis and breakdown of the ECM. Overexpression of TGF-β contributes to the dysregulation of the normal homeostasis at multiple levels: it enhances synthesis and deposition of ECM components including collagen and can also alter the balance of matrix metalloproteinases and their inhibitors.

Interestingly, the cytokine IL-7 can downregulate the production of TGF-β in cells from certain specific lineages (see e.g. Dubinett et al., 1993 *J. Immunol.* 151:6670–6680; Dubinett et al., 1995 *J. Natl. Cancer Inst.* 87:593–597; Miller et al., 1993 *Blood.* 82:3686–3694). This 25-kDa glycoprotein was originally isolated from bone marrow stroma cells as a pre-B lymphocyte growth factor and was subsequently found to augment the growth of T lymphocytes. IL-7 can also potently enhance T cell function and IFN-γ production (see e.g. Miller, P.W., et al. 2000 *Hum. Gene Ther.* 11:53–65; Borger et al., 1996 *J. Immunol.* 156:1333–1338; Mehrotra et al., 1995 *J. Immunol.* 154:5093–5102; Armitage et al., 1992 *Cytokine.* 4:461–469; and Armitage et al., 1990 *J. Immunol.* 144:938–941). In addition, IL-7 synergizes with IL-12 in the induction of T cell proliferation, cytotoxicity, and IFN-γ release and IL-7 plays a role in cell-mediated immune responses characteristic of type 1 cytokines (see e.g. Borger et al., 1996 *J. Immunol.* 156:1333–1338).

The effects of IL-7 in cells of pathologies such as idiopathic pulmonary fibrosis have not been examined previously.

There is a need in the art for novel therapeutic strategies in the treatment of idiopathic pulmonary fibrosis. In addition, there is a need for models based on an better understanding of the physiological aspects of idiopathic pulmonary fibrosis so that new methods and agents can be assessed in contexts that are consistent with phenomena observed with the clinical syndrome. The disclosure provided herein meets these needs.

Summary of the Invention

The invention is based on the finding that the cytokine interleukin-7 (IL-7) limits the development of pulmonary fibrosis in clinically relevant models of idiopathic pulmonary fibrosis (IPF). The disclosure provided herein illustrates how IL-7 can be used to limit the development of pulmonary fibrosis and demonstrates its therapeutic potential in the treatment of idiopathic pulmonary fibrosis. As illustrated below, the disclosure provides additional information relating to the specific cellular mechanisms involved in this pathology and therefore provides models for the assessment of different therapeutic modalities employing IL-7 as well as the assessment of new therapeutic agents and methods.

The invention disclosed herein has a number of embodiments. A preferred embodiment of the invention is a method of treating idiopathic pulmonary fibrosis in a mammalian subject by administering a therapeutically effective amount of IL-7 to the subject. Preferably the IL-7 is human IL-7 such as the IL-7 having the polypeptide sequence shown in SEQ ID NO: 1. In such embodiments the IL-7 can be administered by any one of methods known in the art, preferably by one of the methods typically used to administer agents to the lungs, for example, through the use of a nebulizer, atomizer, inhaler or the like. Most preferably the IL-7 is administered to the subject via a nebulizer.

The IL-7 formulations of the invention can include IL-7 polypeptides conjugated to a polyol such as polyethylene glycol and typically comprise a pharmaceutically

acceptable carrier. In preferred embodiments of the invention, the IL-7 formulations also include a liposome composition including those having lipids such dimimyristol phosphatidyl choline (DMPC). Such liposome compositions are preferred embodiments of the invention due to material characteristics of the liposomes such as being in a solidified form when refrigerated or nebulized at ambient room temperature but in a liquid form when at body temperature. Preferably the IL-7 is the IL-7 polypeptide shown in SEQ ID NO: 1. Alternatively the formulation administered to the mammalian subject includes a vector encoding the IL-7 sequence shown in SEQ ID NO: 1, with the vector being administered so that the cells of the mammal are transduced by the vector and subsequently express IL-7 in an amount sufficient to, for example, inhibit TGF-β production or signaling in the appropriate physiological context (e.g. in the lung of an individual suffering from idiopathic pulmonary fibrosis).

Other embodiments of the invention include methods utilizing IL-7 and an additional agent to effect a physiological process in a pulmonary fibrosis fibroblast. A typical embodiment of such methods is a method of treating interstitial pulmonary fibrosis in a mammalian subject by administering a therapeutically effective amount of the IL-7 polypeptide shown in SEQ ID NO: 1 and an amount of interferon-γ sufficient to increase the expression of Smad7 in a pulmonary fibrosis fibroblast in the subject. Such methods can include additional or alternative steps such as administering an amount of an additional agent such as an anti–TGF-β antibody (see, e.g. J Immunol 1999 Nov 15;163(10):5693-9), a soluble TGF-β receptor (see, e.g. Thorax 1999 Sep;54(9):805-12), or decorin (see, e.g. Am J Physiol Lung Cell Mol Physiol 2001 Jun;280(6):L1327-34) with the agent being added in an amount sufficient to inhibit TGF-β induced collagen synthesis in a pulmonary fibrosis fibroblasts in the subject.

Related embodiments of the invention involve the administration of an amount of IL-7 sufficient to effect on one or more of the biological processes that are identified herein as being associated with TGF-β's role in pathological conditions such as idiopathic pulmonary fibrosis. One such embodiment is a method of treating idiopathic

pulmonary fibrosis in a mammalian subject comprising administering an amount of IL-7 having the sequence shown in SEQ ID NO: 1 to the subject, wherein the amount of IL-7 is sufficient to decrease TGF-β polypeptide production in a pulmonary fibrosis fibroblast in the subject. Another embodiment of the invention is a method of inhibiting TGF-β signaling in a pulmonary fibrosis fibroblast in a IFN-γ independent manner comprising exposing the pulmonary fibrosis fibroblast to an amount of IL-7 sufficient to sufficient to increase the expression of Smad7 in the pulmonary fibrosis fibroblast. Yet another related embodiment of the invention is a method of inhibiting the expression of TGF-β polypeptide in a pulmonary fibrosis fibroblast comprising exposing the pulmonary fibrosis fibroblast to an amount of IL-7 sufficient to inhibit the phosphorylation of Smad3 in the pulmonary fibrosis fibroblast. Yet another embodiment of the invention is a method of inhibiting TGF-β induced collagen synthesis in a pulmonary fibrosis fibroblast comprising exposing the pulmonary fibrosis fibroblast to an amount of IL-7 sufficient to inhibit the expression of TGF-β polypeptide in the pulmonary fibrosis fibroblast.

The disclosure provided herein further provides cell based assays which can be used to evaluate how various IL-7 formulations impact specific physiological processes in pulmonary fibrosis fibroblasts that are associated with a pathology such as idiopathic pulmonary fibrosis (e.g. the JAK1/STAT1-dependent pathway). Related embodiments of the invention are cell based assays which can be used to dissect specific physiological aspects of pathological conditions such as idiopathic pulmonary fibrosis. Related embodiments of the invention are cell based assays which can be used to evaluate how additional agents may effect such processes. One such embodiment of the invention is a method of identifying an agent capable of modulating a TGF-β associated process in a pulmonary fibrosis fibroblast by exposing the pulmonary fibrosis fibroblast to an amount of IL-7 and additionally exposing the pulmonary fibrosis fibroblast to a small molecule or polypeptide agent and observing the agent's effect on a TGF-β associated process such as the inhibition of TGF-β signaling. In an exemplary embodiment of the invention the

agent is a polypeptide selected from the group consisting of interferon-γ, anti–TGF-β antibody and soluble TGF-β receptor.

Brief Description of the Figures

Figures 1a-1d. IL-7 decreases TGF-β production by PFF in an IFN-γ–independent manner. (**1a** and **1b**) Primary fibroblasts from IPF patients (PFF, $n = 5$) or non-IPF subjects (NF, $n = 5$) were cultured in medium with or without IL-7 for 24 hours, and the culture supernatants were assayed for TGF-β by ELISA. IL-7 decreases TGF-β production in PFF, but not in NF. (**1c** and **1d**). While IFN-γ decreased TGF-β production in both NF and PFF, IL-7 decreased TGF-β production only in PFF. The addition of anti–IFN-γ antibody did not alter the capacity of IL-7 to decrease TGF-β, providing evidence that IL-7 acts in an IFN-γ–independent manner. $*P < 0.01$.

Figure 2. IL-7R signaling requires an intact JAK1/STAT1 pathway. U4A, U3A, and U4A/JAK1 fibroblast cell lines were cultured in medium containing variable doses of recombinant IL-7 for a 24-hour incubation. Culture supernatants were assayed for TGF-β by ELISA. Whereas TGF-β production remained unchanged in the U4A and U3A cells, IL-7 decreased TGF-β production in a dose-dependent manner in U4A/JAK1 cells ($P < 0.01$ at 50–400 ng/ml IL-7).

Figure 3. IL-7 inhibits TGF-β–induced collagen synthesis in PFF. PFF ($n = 3$) and NF ($n = 4$) were cultured in medium alone, medium containing TGF-β1, or the combination of TGF-β1 plus IL-7 for 24 hours. The collagen content from the cell lysates was determined. IL-7 inhibits TGF-β–induced collagen synthesis in primary PFF but not in NF. $*P < 0.01$.

Figures 4a and 4b. IL-7 inhibits TGF-β signaling through the induction of Smad7. (**4a**) The Smad7 expression from PFF ($n = 3$) or NF ($n = 3$) was determined by RT-PCR. The upper panel shows amplified Smad7 PCR product; the lower panel shows GAPDH expression in the same samples. Lanes 1–3 and 7–9 are NF. Lanes 4–6 and 10–12 are PFF. Lanes 1–6: Fibroblasts were cultured in medium alone. Lanes 7–12: Fibroblasts were incubated in IL-7 for 24 hours. M, molecular weight marker. IL-7

increases Smad7 mRNA expression in both NF and PFF. (**4b**) PFF and Smad7DN-transduced PFF were incubated in medium alone, medium containing TGF-β1, or medium containing TGF-β1 plus IL-7 for a 24-hour incubation. IL-7–mediated inhibition of collagen synthesis by TGF-β was not observed in Smad7DN-transduced PFF. $*P < 0.01$.

Figures 5a-5d. IL-7 inhibits bleomycin-induced pulmonary fibrosis in vivo. Lung homogenates from each group of mice ($n = 10$) were assayed for hydroxyproline (**5a**), collagen (**5b**), TGF-β (**5c**), and IFN-γ (**5d**) content. The data represent the average values of each group of mice with standard deviation. IL-7 decreases hydroxyproline and collagen levels, decreases TGF-β, and increases IFN-γ in bleomycin-treated lung homogenates. $*P < 0.01$ IL-7 + bleomycin compared with bleomycin alone.

Figures 6a and 6b. IL-7–mediated antifibrotic activities are IFN-γ–independent. Lung homogenates from each group of mice ($n = 10$) were assayed for hydroxyproline (**6a**) and collagen (**6b**). The data represent the average values of each group of mice with standard deviation. IL-7 decreases hydroxyproline and collagen levels independent of IFN-γ in bleomycin-treated lung homogenates. $*P < 0.01$ IL-7 + bleomycin compared with bleomycin alone.

Detailed Description of the Invention

Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the techniques and procedures described or referenced herein are well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in see Ausubel et al., Current Protocols in Molecular Biology, Wiley

Interscience Publishers, (1995) and Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd. edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

A. Brief Characterization of Features of the Invention

The invention is based on the discoveries disclosed herein that the cytokine interleukin-7 (IL-7) limits the development of pulmonary fibrosis in clinically relevant models of idiopathic pulmonary fibrosis (IPF). The disclosure provided herein illustrates how IL-7 can be used to limit the development of pulmonary fibrosis and demonstrates its therapeutic potential in the treatment of idiopathic pulmonary fibrosis. As illustrated below, the disclosure provides additional information relating to the cellular mechanisms involved in this pathology and provides novel models for the assessment of new therapeutic agents and methods.

The invention described herein is based in part on the discovery that recombinant IL-7 limits the development of pulmonary fibrosis in a murine model of idiopathic pulmonary fibrosis. In this context, IL-7 is shown to inhibit TGF-β production and signaling in pulmonary fibrosis fibroblasts (PFF), a finding which is consistent with the role that TGF-β plays as a critical fibrogenic factor in the development of pulmonary fibrosis. This discovery is significant for a number of reasons, including the fact that IL-7 has distinct advantages over conventional agents used in the treatment of IPF. For example, in marked contrast to the conventional IPF therapeutic regimens that use steroids and immunosuppressive agents, IL-7 potently enhances cell-mediated immunity.

The disclosure provided herein also characterizes various physiological processes associated with IPF. As is documented for several other cytokines, IL-7 signals via the JAK1/STAT1–dependent pathway in lymphocytes (see e.g. van der Plas, D.C., et al. 1996 *Leukemia.* **10**:1317–1325). As shown herein, JAK1/STAT1–dependent signaling is

also operative for IL-7 in human fibroblasts. JAK/STAT–dependent signaling in fibroblasts has been previously found to be critical for the capacity of IFN-γ to upregulate Smad7 and inhibit TGF-β signaling (see e.g. Ulloa, L., Doody, J., and Massague, J. 1999 *Nature.* **397**:710–713). IFN-γ signaling rapidly increases the expression of Smad7 (a major inhibitory regulator in the SMAD family) causing the inhibition of Smad3 phosphorylation and subsequently loss of TGF-β signaling to the nucleus (see e.g. Ulloa, L., Doody, J., and Massague, J. 1999 *Nature.* **397**:710–713; Massague, J. 1998 *Annu. Rev. Biochem.* **67**:753–791). These findings indicate a mechanism of transmodulation between the STAT and SMAD signal transduction pathways.

In addition to the capacity to downregulate TGF-β production in pulmonary fibrosis fibroblasts, IL-7 can potently induce Smad7 and subsequently block TGF-β signaling. Moreover, this IL-7–mediated inhibition of TGF-β signaling is associated with an increase in Smad7. In the presence of IL-7, Smad7 dominant negative fibroblasts restored TGF-β–induced collagen synthesis, indicating that an IL-7–mediated increase in Smad7 suppresses TGF-β signaling.

The functional manifestation of this blockade in TGF-β signaling is demonstrated by a profound decrease in the TGF-β-induced collagen synthesis in PFF. IFN-γ is known to decrease both TGF-β production and signaling and has shown promise in preliminary clinical trials (see e.g. DuBois et al., 1999 *N. Engl. J. Med.* **341**:1302–1304). Although IL-7 is known to induce IFN-γ our current study indicates that IL-7 mediates potent antifibrotic responses both in vitro and in vivo in an IFN-γ–independent manner.

As is known in the art, a variety of models can be to assess potential therapeutic interventions (e.g. methods using IL-7) for pulmonary fibrosis (see e.g. Keane, M.P., et al. 1999 *J. Immunol.* **163**:5686–5692; Keane, M.P., et al. 1999 *J. Immunol.* **162**:5511–5518; Keerthisingam, C.B., et al. 2001 *Am. J. Pathol.* **158**:1411–1422; Giri et al., 1993 *Thorax.* **48**:959–966; Zhang et al., 1995 *Am. J. Pathol.* **147**:352–361; Bowden, D.H. 1984 *Lab. Invest.* **50**:487–48; Nakao, A., et al. 1999 *J. Clin. Invest.* **104**:5–11; Krishna, G., et al. 2001

Am. J. Pathol. **158**:997–1004; Hoyt, D.G., and Lazo, J.S. 1992 *Am. J. Respir. Cell Mol. Biol.* **7**:645–651; Christensen et al., 1999 *Am. J. Pathol.* **155**:1773–1779; Wang, Q., et al. 1999 *Thorax.* **54**:805–812; Corbel, M., et al. 2001 *J. Pathol.* **193**:538–545; Giri, S.N., et al. 1997 *Biochem. Pharmacol.* **54**:1205–1216; Kolb, M., et al. 2001 *Am. J. Respir. Crit. Care Med.* **163**:770–777; Gurujeyalakshmi, G., and Giri, S.N. 1995 *Exp. Lung Res.* **21**:791–808; Cooper, J.A., Jr. 2000 *Am. J. Respir. Cell Mol. Biol.* **22**:520–523). Such models can be readily adapted to examine the effect of IL-7 on physiological processes associated with IPF in the various contexts provided by the different models.

In the study disclosed herein the bleomycin model is a preferred model for examining IPF due to its widespread use for this purpose and because bleomycin induces a TGF-β dependent induction of pulmonary fibrosis that is accompanied by increased collagen and hydroxyproline synthesis (see e.g. Keane, M.P., et al. 1999 *J. Immunol.* **163**:5686–5692; Keane, M.P., et al. 1999 *J. Immunol.* **162**:5511–5518; Keerthisingam, C.B., et al. 2001 *Am. J. Pathol.* **158**:1411–1422; Giri et al., 1993 *Thorax.* **48**:959–966; Zhang et al., 1995 *Am. J. Pathol.* **147**:352–361; Bowden, D.H. 1984 *Lab. Invest.* **50**:487–488; Nakao, A., et al. 1999 *J. Clin. Invest.* **104**:5–11; Krishna, G., et al. 2001 *Am. J. Pathol.* **158**:997–1004; Hoyt, D.G., and Lazo, J.S. 1992 *Am. J. Respir. Cell Mol. Biol.* **7**:645–651; Wang, Q., et al. 1999 *Thorax.* **54**:805–812; Corbel, M., et al. 2001 *J. Pathol.* **193**:538–545; Giri, S.N., et al. 1997 *Biochem. Pharmacol.* **54**:1205–1216; Kolb, M., et al. 2001 *Am. J. Respir. Crit. Care Med.* **163**:770–777; Gurujeyalakshmi, G., and Giri, S.N. 1995 *Exp. Lung Res.* **21**:791–808; Cooper, J.A., Jr. 2000 *Am. J. Respir. Cell Mol. Biol.* **22**:520–523). This model is also preferred due to studies indicating that administration of anti–TGF-β antibodies, soluble TGF-β receptors, the TGF-β inhibitor decorin, or Smad7 gene transfer limits bleomycin-induced pulmonary fibrosis (see e.g. Giri, S.N., et al. 1997 *Biochem. Pharmacol.* **54**:1205–1216; Kolb, M., et al. 2001 *Am. J. Respir. Crit. Care Med.* **163**:770–777; Wang, Q., et al. 1999 *Thorax.* **54**:805–812; Giri et al., 1993 *Thorax.* **48**:959–966; Nakao, A., et al. 1999 *J. Clin. Invest.* **104**:5–11).

Consistent with our in vivo findings in the murine bleomycin model, IL-7 decreases TGF-β production in in vitro in human fibroblasts derived from pulmonary

fibrosis biopsies (see, e.g. Example 2 below). Although investigators have shown that TGF-β can impact the phenotype and function of normal fibroblasts, our studies also demonstrate marked differences between normal and fibrosis fibroblasts (see e.g. Ramos, C., et al. 2001 *Am. J. Respir. Cell Mol. Biol.* **24**:591–598; Reisdorf et al., 2001 *Am. J. Pathol.* **159**:226–272; Chin, G.S., et al. 2001 *Plast. Reconstr. Surg.* **108**:423–429; Kishi et al., 1999 *Br. J. Plast. Surg.* **52**:579–582; and Hakenjos et al., 2000 *Int. J. Radiat. Biol.* **76**:503–509).

Surprisingly, the physiological effects of IL-7 that are observed in pulmonary fibrosis fibroblasts (PFF) are different from those observed in normal fibroblasts (NF). In particular, the disclosure provided herein further shows that recombinant IL-7 significantly decreases TGF-β production in pulmonary fibrosis fibroblasts but not in normal fibroblasts. Because IL-7R expression does not differ between PFF and NF cells, downstream regulatory signaling events likely account for this difference in IL-7 responsiveness. The differences in TGF-β induced collagen synthesis by NF and PFF may be due to a differential response of these cell populations to this cytokine (see e.g. Ramos, C., et al. 2001 *Am. J. Respir. Cell Mol. Biol.* **24**:591–598; Reisdorf et al., 2001 *Am. J. Pathol.* **159**:226–272; Chin, G.S., et al. 2001 *Plast. Reconstr. Surg.* **108**:423–429; Kishi et al., 1999 *Br. J. Plast. Surg.* **52**:579–582; and Hakenjos et al., 2000 *Int. J. Radiat. Biol.* **76**:503–509).

As shown in the Examples below, recombinant TGF-β does not affect collagen synthesis in normal fibroblasts. Because activated recombinant TGF-β1 was used in these studies, potential differences in TGF-β activation state could not account for these observations. These findings are consistent with previous investigations that revealed marked differences in TGF-β dependent responses in fibroblasts from different developmental or pathogenic states (see e.g. Reisdorf et al., 2001 *Am. J. Pathol.* **159**:226–272; Chin, G.S., et al. 2001 *Plast. Reconstr. Surg.* **108**:423–429; Kishi et al., 1999 *Br. J. Plast. Surg.* **52**:579–582; and Hakenjos et al., 2000 *Int. J. Radiat. Biol.* **76**:503–509).

The disclosure provided herein illustrates novel therapeutic strategies in the treatment of idiopathic pulmonary fibrosis. In addition, the disclosure provides models

for a detailed examination of various physiological aspects of idiopathic pulmonary fibrosis which allows new methods and agents to be assessed in contexts that are consistent with phenomena observed with this clinical syndrome.

B. Typical Methodologies for Practicing Embodiments of the Invention

The methods disclosed herein may be employed in protocols for treating pathological conditions in mammals such as idiopathic pulmonary fibrosis. In typical methods, IL-7 polypeptide is administered to a mammal, alone or in combination with still other therapeutic agents or techniques. Diagnosis in mammals of the various pathological conditions described herein can be made by the skilled practitioner. A variety of diagnostic techniques are known in the art which allow the diagnosis or detection of idiopathic pulmonary fibrosis in a mammal.

Polypeptides useful in the methods of the invention encompass both naturally occurring proteins as well as variations and modified forms thereof. As noted above, "IL-7 polypeptide or protein" is meant an interleukin-7. IL-7 includes naturally occurring mammalian IL-7s, and variants and fragments thereof, as defined below. Human and murine IL-7 have been cloned and are well known in the art (see, e.g. GenBank Accession Nos.: P13232 and NP_032397 respectively). Preferably the IL-7 is of human or mouse origin (see, e.g. SEQ ID NOS: 1 and 2 in Table 1 respectively). Most preferably the IL-7 is human IL-7.

IL-7 polypeptides for use in the methods disclosed herein can be IL-7 variants, IL-7 fragments, analogues, and derivatives. By "analogues" is intended analogues of either IL-7 or an IL-7 fragment that comprise a native IL-7 sequence and structure, having one or more amino acid substitutions, insertions, or deletions. Peptides having one; or more peptoids (peptide mimics) are also encompassed by the term analogues (WO 91/04282). By "derivatives" is intended any suitable modification of IL-7, IL-7 fragments, or their respective analogues, such as glycosylation, phosphorylation, or other addition of foreign moieties (e.g. Pegylation as described below), so long as the desired

activity is retained. Methods for masking IL-7 fragments, analogues, and derivatives are available in the art.

As used herein, the IL-7 gene and IL-7 protein includes the murine and human IL-7 genes and proteins specifically described herein, as well as biologically active structurally and/or functionally similar variants or analog of the foregoing. IL-7 peptide analogs generally share at least about 50%, 60%, 70%, 80%, 90% or more amino acid homology (using BLAST criteria). For example, % identity values may be generated by WU-BLAST-2 (Altschul et al., 1996, Methods in Enzymology 266:460-480; http://blast.wustl/edu/blast/README.html). IL-7 nucleotide analogs preferably share 50%, 60%, 70%, 80%, 90% or more nucleic acid homology (using BLAST criteria). In some embodiments, however, lower homology is preferred so as to select preferred residues in view of species-specific codon preferences and/or optimal peptide epitopes tailored to a particular target population, as is appreciated by those skilled in the art. Fusion proteins that combine parts of different IL-7 proteins or fragments thereof, as well as fusion proteins of a IL-7 protein and a heterologous polypeptide are also included. Such IL-7 proteins are collectively referred to as the IL-7-related proteins, the proteins of the invention, or IL-7.

The term "variant" refers to a molecule that exhibits a variation from a described type or norm, such as a protein that has one or more different amino acid residues in the corresponding position(s) of a specifically described protein. An analog is an example of a variant protein. As used herein, the IL-7-related gene and IL-7-related protein includes the IL-7 genes and proteins specifically described herein, as well as structurally and/or functionally similar variants or analog of the foregoing. IL-7 peptide analogs generally share at least about 50%, 60%, 70%, 80%, 90% or more amino acid homology (using BLAST criteria). IL-7 nucleotide analogs preferably share 50%, 60%, 70%, 80%, 90% or more nucleic acid homology (using BLAST criteria). In some embodiments, however, lower homology is preferred so as to select preferred residues in view of species-specific codon preferences and/or optimal peptide epitopes tailored to a particular target population, as is appreciated by those skilled in the art.

Embodiments of the invention disclosed herein include a wide variety of art-accepted variants or analogs of IL-7 proteins such as polypeptides having amino acid insertions, deletions and substitutions. IL-7 variants can be made using methods known in the art such as site-directed mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis (Carter et al., *Nucl. Acids Res., 13*:4331 (1986); Zoller et al., *Nucl. Acids Res., 10*:6487 (1987)), cassette mutagenesis (Wells et al., Gene, 34:315 (1985)), restriction selection mutagenesis (Wells et al., *Philos. Trans. R. Soc. London SerA*, 317:415 (1986)) or other known techniques can be performed on the cloned DNA to produce the IL-7 variant DNA. Resulting mutants can be tested for biological activity. Sites critical for binding can be; determined by structural analysis such as crystallization, photoaffinity labeling, or nuclear magnetic resonance. See, deVos et al. (1992) Science 255:306 and Smith et al. (1992:) J. Mol. Biol. 224:899.

As is known in the art, conservative amino acid substitutions can frequently be made in a protein without altering the functional activity of the protein. Proteins of the invention can comprise conservative substitutions. Such changes typically include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa. Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine (A) and valine (V). Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence that is involved in a specific biological activity

such as a protein-protein interaction. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions (Creighton, *The Proteins,* (W.H. Freeman & Co., N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)). If alanine substitution does not yield adequate amounts of variant, an isosteric amino acid can be used.

Variant IL-7 proteins and IL-7 polypeptide fragments useful in the methods of the present invention must possess IL-7 biological activity. Specifically, they must possess the desired biological activity of the native protein, for example the ability to inhibit TGF-β production and signaling in pulmonary fibrosis fibroblasts, the ability to increase the expression Smad7 (and/or inhibit the phosphorylation of Smad3), the ability to inhibit TGF-β induced collagen synthesis or one of the other activities described herein. For the purposes of the invention, a "IL-7 variant"' will exhibit at least 30% of the activity of the IL-7 shown in SEQ ID NO: 1 or SEQ ID NO: 2 (e.g. limits the development of pulmonary fibrosis in a clinically relevant of idiopathic pulmonary fibrosis). More typically, variants exhibit more than 60% of at least one of these activities; even more typically, variants exhibit more than 80% of at least one of these activities.

The description below also provides methods of producing IL-7 covalently attached (hereinafter "conjugated") to one or more chemical groups. Chemical groups suitable for use in an IL-7 conjugate of the present invention are preferably not significantly toxic or immunogenic. The chemical group is optionally selected to produce an IL-7 conjugate that can be stored and used under conditions suitable for storage. A variety of exemplary chemical groups that can be conjugated to polypeptides are known in the art and include for example carbohydrates, such as those carbohydrates that occur

naturally on glycoproteins, and non-proteinaceous polymers, such as polyols (see, e.g., U.S. Patent No. 6,245,901).

A polyol, for example, can be conjugated to polypeptides such as an IL-7 at one or more amino acid residues, including lysine residues, as is disclosed in WO 93/00109. The polyol employed can be any water-soluble poly(alkylene oxide) polymer and can have a linear or branched chain. Suitable polyols include those substituted at one or more hydroxyl positions with a chemical group, such as an alkyl group having between one and four carbons. Typically, the polyol is a poly(alkylene glycol), such as poly(ethylene glycol) (PEG), and thus, for ease of description, the remainder of the discussion relates to an exemplary embodiment wherein the polyol employed is PEG and the process of conjugating the polyol to a polypeptide is termed "pegylation." However, those skilled in the art recognize that other polyols, such as, for example, poly(propylene glycol) and polyethylene-polypropylene glycol copolymers, can be employed using the techniques for conjugation described herein for PEG. Illustrative examples of cytokines conjugated with PEG are shown, for example, in U.S. Patent No. 5,795,569; U.S. Patent No. 4,902,502; Wang et al., Biochemistry 2000, 39, 10634-10640; Leong et al., Cytokine 2001, 16(3): 24-36; and Kozlowski et al., BioDrugs 2001; 15(7): 419-429.

The average molecular weight of the PEG employed in the pegylation of the IL-7 can vary, and typically may range from about 500 to about 30,000 daltons (D). Preferably, the average molecular weight of the PEG is from about 1,000 to about 25,000 D, and more preferably from about 2,000 to about 5,000 D. In one embodiment, pegylation is carried out with PEG having an average molecular weight of about 2,000 D. Preferably, the PEG homopolymer is unsubstituted, but it may also be substituted at one end with an alkyl group. Preferably, the alkyl group is a C1-C4 alkyl group, and most preferably a methyl group. PEG preparations are commercially available, and typically, those PEG preparations suitable for use in the present invention are nonhomogeneous preparations sold according to average molecular weight. For example, commercially available PEG(5000) preparations typically contain molecules that vary slightly in molecular weight, usually ± 500 D.

A variety of methods for pegylating proteins are known in the art. Specific methods of producing proteins conjugated to PEG include the methods described in U.S. Pat. No. 4,179,337, U.S. Pat. No. 4,935,465, U.S. Patent No. 5,824,784 and U.S. Patent No. 5,849,535. Typically the protein is covalently bonded via one or more of the amino acid residues of the protein to a terminal reactive group on the polymer, depending mainly on the reaction conditions, the molecular weight of the polymer, etc. The polymer with the reactive group(s) is designated herein as activated polymer. The reactive group selectively reacts with free amino or other reactive groups on the protein. The PEG polymer can be coupled to the amino or other reactive group on the protein in either a random or a site specific manner. It will be understood, however, that the type and amount of the reactive group chosen, as well as the type of polymer employed, to obtain optimum results, will depend on the particular protein or protein variant employed to avoid having the reactive group react with too many particularly active groups on the protein. As this may not be possible to avoid completely, it is recommended that generally from about 0.1 to 1000 moles, preferably 2 to 200 moles, of activated polymer per mole of protein, depending on protein concentration, is employed. The final amount of activated polymer per mole of protein is a balance to maintain optimum activity, while at the same time optimizing, if possible, the circulatory half-life of the protein and/or the solubility of the protein (see, e.g. U.S. Patent No. 4,766,106).

While the residues may be any reactive amino acids on the protein, such as the N-terminal amino acid group, preferably the reactive amino acid is cysteine, which is linked to the reactive group of the activated polymer through its free thiol group as shown, for example, in W0 99/03887, WO 94/12219, WO 94/22466, U.S. Patent No. 5,766,897, U.S. Patent No. 5,206,344, U.S. Patent No. 5,166,322, and U.S. Patent No. 5,206,344. Alternatively the reactive group is lysine, which is linked to the reactive group of the activated polymer through its free epsilon-amino group, or glutamic or aspartic acid, which is linked to the polymer through an amide bond. This reactive group can then react with, for example, the α and ε amines of proteins to form a covalent bond. Conveniently, the other end of the PEG molecule can be "blocked" with a non-reactive

chemical group, such as a methoxy group, to reduce the formation of PEG-crosslinked complexes of protein molecules.

Suitable activated PEGs can be produced by a number of conventional reactions. For example, a N-hydroxysuccinimide ester of a PEG (M-NHS-PEG) can be prepared from PEG-monomethyl ether (which is commercially available from Union Carbide) by reaction with N,N'-dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide (NHS), according to the method of Buckmann and Merr, Makromol. Chem., 182:1379-1384 (1981). In addition, a PEG terminal hydroxy group can be converted to an amino group, for example, by reaction with thionyl bromide to form PEG-Br, followed by aminolysis with excess ammonia to form PEG-NH_2. The PEG-NH_2 is then conjugated to the protein of interest using standard coupling reagents, such as Woodward's Reagent K. Furthermore, a PEG terminal $-CH_2$ OH group can be converted to an aldehyde group, for example, by oxidation with MnO_2. The aldehyde group is conjugated to the protein by reductive alkylation with a reagent such as cyanoborohydride. Alternatively, activated PEGs suitable for use in the present invention can be purchased from a number of vendors. For example, Shearwater Polymers, Inc. (Huntsville, Ala.) sells methoxy-PEG-maleimide, MW 2,000, in addition to a succinimidyl carbonate of methoxy-PEG and methoxy-PEG succinimidyl propionate.

The degree of pegylation of IL-7 of the present invention can be adjusted to provide a desirably increased *in vivo* half-life (hereinafter "half-life"), compared to the corresponding non-pegylated IL-7. It is believed that the half-life of a pegylated IL-7 typically increases incrementally with increasing degree of pegylation. The degree and sites of pegylation of a protein are determined, e.g., by (1) the number and reactivities of pegylation sites (e.g., primary amines) and (2) pegylation reaction conditions. As some of the pegylation sites in a protein are likely to be relatively unreactive, standard pegylation reactions typically result in less than complete pegylation.

Standard mutagenesis techniques can be used to alter the number of potential pegylation sites in a protein. Thus, to the extent that amino acid substitutions introduce or replace amino acids such as cysteine and lysine, IL-7 of the present invention can

contain a greater or lesser number of potential pegylation sites than native sequence IL-7 (shown in Table 1). The degree and sites of pegylation can also be manipulated by adjusting reaction conditions, such as the relative concentrations of the activated PEG and the protein as well as the pH. Suitable conditions for a desired degree of pegylation can be determined empirically by varying the parameters of standard pegylation reactions.

Pegylated proteins can be characterized by SDS-PAGE, gel filtration, NMR, peptide mapping, liquid chromatography-mass spectrophotometry, and *in vitro* biological assays. The extent of pegylation is typically first shown by SDS-PAGE. Polyacrylamide gel electrophoresis in 10% SDS is typically run in 10 mM Tris-HCl pH 8.0, 100 mM NaCl as elution buffer. To demonstrate which residue is pegylated, peptide mapping using proteases such as trypsin and Lys-C protease can be performed. Thus, samples of pegylated and non-pegylated peptides can be digested with a protease such as Lys-C protease and the resulting peptides separated by a technique such as reverse phase HPLC. The chromatographic pattern of peptides produced can be compared to a peptide map previously determined for the polypeptide. Each peak can then be analyzed by mass spectrometry to verify the size of the fragment in the peak. The fragment(s) that carried PEG groups are usually not retained on the HPLC column after injection and disappear from the chromatograph. Such disappearance from the chromatograph is an indication of pegylation on that particular fragment that should contain at least one pegylatable amino acid residue.

The IL-7 may be administered directly by introducing a IL-7 polypeptide, IL-7 variant or IL-7 fragment into or onto the subject. Alternatively, the IL-7 may be produced in situ following the administration of a polynucleotide encoding a IL-7 polypeptide, IL-7 variant or IL-7 fragment may be introduced into the subject.

Polynucleotides for use in the methods disclosed herein may be naturally occurring, such as allelic variants, homologs, orthologs, or may be constructed by recombinant DNA methods or by chemical synthesis. Alternatively, the variant polypeptides may be non-naturally occurring and made by techniques known in the art,

including mutagenesis. Polynucleotide variants may contain nucleotide substitutions, deletions, inversions and insertions.

IL-7 encoding nucleic acid molecules can be inserted into vectors and used as gene therapy vectors. In this context, a wide range of other host-vector systems suitable for the expression of IL-7 proteins or fragments thereof are known in the art, see for example, Sambrook et al., 1989, Current Protocols in Molecular Biology, 1995, supra. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (U.S. 5,328,470), implantation or by stereotactic injection (see e.g., Chen et al., PNAS 91:3054-3057 (1994)). Vectors for expression in mammalian hosts are disclosed in Wu et al. (1991) J. Biol. Chem. 266:14338; Wu and Wu (1988) J. Biol. Chem. 263:14621; and Zenke et al. (1990) Proc. Nat'l. Acad Sci. USA 87:3655. The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g. retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system. Preferred for use in the present invention are adenovirus vectors. These vectors may be employed to deliver and express a wide variety of genes, including, but not limited to cytokine genes such as those of the interferon gene family and the interleukin gene family. A preferred method for delivery of the expression constructs involves the use of an adenovirus expression vector. Although adenovirus vectors are known to have a low capacity for integration into genomic DNA, this feature is counterbalanced by the high efficiency of gene transfer afforded by these vectors. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct in host cells with complementary packaging functions and (b) to ultimately express a heterologous gene of interest that has been cloned therein.

The IL-7 polypeptides, IL-7 polypeptide variants, IL-7 polypeptide fragments, IL-7 polynucleotides encoding said polypeptides, variants and fragments, and the IL-7 agents useful in the methods of the invention can be incorporated into pharmaceutical

compositions suitable for administration into a mammal. The term "mammal" as used herein refers to any mammal classified as a mammal, including humans, mice, cows, horses, dogs and cats. In a preferred embodiment of the invention, the mammal is a human. Such compositions typically comprise at least one IL-7 polypeptide, IL-7 polypeptide variant, IL-7 polypeptide fragment, IL-7 polynucleotide encoding said polypeptide, variant or fragment, an IL-7 agent, or a combination thereof, and a pharmaceutically acceptable carrier. Methods for formulating the IL-7 compounds of the invention for pharmaceutical administration are known to those of skill in the art. See, for example, Remington: The Science and Practice of Pharmacy, 19th Edition, Gennaro (ed.) 1995, Mack Publishing Company, Easton, PA.

As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, such media can be used in the compositions of the invention. Supplementary active compounds can also be incorporated into the compositions. A pharmaceutical composition of the, invention is formulated to be compatible with its intended route of administration.

The pharmaceutical compositions of the invention, comprising IL-7 polypeptides, IL-7 polypeptide variants, IL-7 polypeptide fragments, polynucleotides encoding said IL-7 polypeptides, variants and fragments, as well as IL-7 agents, as defined above, are administered in therapeutically effective amounts. The "therapeutically effective amount" refers to a nontoxic dosage level sufficient to induce a desired biological result (e.g. a diminution of the severity of the symptoms associated with a pathological condition such as IPF). Amounts for administration may vary based upon the desired activity, the diseased state of the mammal being treated, the dosage form, method of administration, patient factors such as age, sex, and severity of disease.

It is recognized that a therapeutically effective amount is provided in a broad range of concentrations. Such range can be determined based on in vitro and/or in vivo assays.

Therapeutic compositions of the IL-7 can be prepared by mixing the desired IL-7 molecule having the appropriate degree of purity with optional pharmaceutically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A. ed. (1980)), in the form of lyophilized formulations, aqueous solutions or aqueous suspensions. Acceptable carriers, excipients, or stabilizers are preferably nontoxic to recipients at the dosages and concentrations employed, and include buffers such as Tris, HEPES, PIPES, phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

Additional examples of such carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts, or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, and cellulose-based substances. Carriers for topical or gel-based forms include polysaccharides such as sodium carboxymethylcellulose or methylcellulose, polyvinylpyrrolidone, polyacrylates, polyoxyethylene-polyoxypropylene-block polymers, polyethylene glycol, and wood wax

alcohols. For all administrations, conventional depot forms are suitably used. Such forms include, for example, microcapsules, nano-capsules, liposomes, plasters, inhalation forms, nose sprays, sublingual tablets, and sustained-release preparations.

Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing, for example, the IL-7 polypeptide, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of IL-7 polypeptide being administered.

Solutions or suspensions used for administering IL-7 can include the following components: a sterile diluent such as water for injection, saline solution; fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. In one embodiment, a pharmaceutical composition can be delivered via slow release formulation or matrix comprising IL-7 protein or DNA constructs suitable for expression of IL-7 protein into or around a site within the body.

A variety of specific formulations that are specifically designed for the administration of cytokines such as IL-7 are known in the art, with different formulation parameters effecting the function of the agent so administered. For example, formulations including pegylated cytokines that complexed with bioadhesive polymers suitable for topical administration to mucosal tissues are described in U.S. Patent No. 6,165,509. As is known in the art, formulations can also be manipulated to modulate the timed release of the therapeutic agent. For example, U.S. Patent No. 5,102,872 discloses the administration of a microencapsulated composition comprising an interleukin conjugated with a polyoxyethylene polymer, and mixed with a release-modulating amount of human serum albumin. In this formulation, the microcapsules are

administered parenterally, and release an effective amount of conjugated interleukin continuously over a period of 14-30 days.

A variety of formulations are known in the art for use in stabilizing a cytokine against the loss of activity. For example, U.S. Patent No. 5,874,075 describes stable compositions of proteins where a protein capable of transitioning into the molten globular state is contacted with a negatively charged lipid vesicle, thereby stabilizing the protein against thermally-induced aggregation, denaturation, and loss of activity. In such formulations, the protein:phospholipid complex can directly stabilize the secondary and tertiary structure of the protein, with the compositions being useful in high temperature formulations and in novel delivery vehicles.

Yet another formulation known in the art to be useful to facilitate the delivery of polypeptides such as IL-7 involves the use of liposomes which can be used to improve cytokine pharmacokinetics, immunomodulatory activity and reduce toxicity (see, e.g. Kendar et al., J. Immunother. 16:47-59, 1994; Kendar et al., 16:115-124, 1994; and Kendar et al., in S. Chouaib (ed.), The Biotherapy of Cancers: From immunotherapy to Gene Therapy, pp. 333-362. Paris: INSERM, 1998). In this context Cabanes et al., Clinical Cancer Research Vol. 5, 687-693 (1999) describe liposomal interleukin formulations having enhanced biological activity.

In a preferred formulation of the invention, an IL-7 formulation is prepared aerosol administration in a manner analogous to that used with IL-2 as described in Ten et al., Int Immunopharmacol 2002 Feb;2(2-3):333-44. In such aerosol formulations, lipids such as the pure, synthetic lipid, dimimyristol phosphatidyl choline (DMPC) are preferred due to characteristics such as a melting temperature of about 23^{0}C wherein the liposomes are solid when refrigerated or nebulized at ambient room temperature and are liquid at 37^{0}C. With the use of such formulations, liposome encapsulation of the interleukin ensures not only correct particle size for nebulization (see, also Khanna et al., Cancer 1997; 79:1409-1421), but also absorption and delivery of the biologically active cytokine. For other typical aerosol formulations of interleukins, see, also Skubitz et al., Anti-Cancer Drugs, 2000; 11:555-563.

As is know in the art, liposomes are also useful in transducing cells with polynucleotides and are therefore useful in embodiments of the invention pertaining to the administration of IL-7 polypeptides. Illustrative liposome-mediated transfection formulations can be found, for example in Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995), units 9 and 16. In addition, a number of methods for enhancing transfection efficiency are known in the art such as those described in Gebrekidan et al., AAPS PharmSciTech, 2000; 1(4) article 28 which describes the use of poly (D, L-lactide-co-glycolide) microspheres containing plasmid DNA for gene delivery. Such compositions can be used to formulate a controlled-release delivery system for DNA that can protect the DNA from DNAse degradation without loss of functional activity.

IL-7 can also be administered in the form of a variety of sustained-release preparations. For example, IL-7 may be delivered to the lung for slow release via encapsulation or carrier materials such as liposomes, or other drug "shells" such as albumin (Albunex by Molecular Biosystems), sugars (Levovist by Schering), gelatins, or lipids. Other suitable examples of sustained-release preparations for use with polypeptides including semipermeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (*e.g.*, poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*, J. Biomed. Mater. Res., 15: 167-277 (1981) and Langer, Chem. Tech., 12: 98-105 (1982) or poly(vinylalcohol)), polylactides (U.S. Patent No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, Biopolymers, 22: 547-556 (1983)), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the Lupron Depot (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

The route of administration may vary depending on the desired effect and/or outcome. Generally for initiation of an IL-7 mediated response, introduction of the IL-7

at or near the desired site of response is utilized. Preferably the IL-7 is administered via an aerosol route. Alternatively additional routes of administration, such as a systemic administration of IL-7, may be employed. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, intramuscular, subcutaneous, oral (e.g., inhalation) transdermal (topical), transmucosal (e.g. a nasal spray), and rectal administration. The IL-7 polypeptide may also be administered by perfusion techniques, such as isolated tissue perfusion, to exert local therapeutic effects. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution; fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. Regimens of administration may vary. A single dose or multiple doses of the agent may be used. Such regimens can vary depending on the severity of the disease and the desired outcome. Following administration of a IL-7 polypeptide to the mammal, the mammal's physiological condition can be monitored in various ways well known to the skilled practitioner familiar with IPF, or alternatively by monitoring the effects of administration of IL-7 on TGF-β production and/or signaling.

In a preferred mode of the invention, a preparation comprising a liquid formulation of IL-7 polypeptide is used. The formulations of the invention can be administered by one of the methods typically used to administer agents to organs such as the lungs. For example, the liquid may be aerosolized for inhalation as a mist via an aerosolization device such as a nebulizer, atomizer, inhaler or the like. Typical methods for use in this context are described in U.S. patent application Serial No. 09/863,849 which is incorporated herein by reference.

In accordance with one mode of the invention, a formulation includes a dry powder which individuals would mix at home or the hospital with saline or water before instillation to an aerosol device. The device would produce an aerosol for inhalation by

the patient. A dry powder formulation could also be delivered in powder form by an aerosol device, such as air gun powered aerosol chamber. Companies which produce dry powder delivery devices include Dura Delivery Systems (the "Dryhaler"), Inhale Therapeutics, and Glaxo Wellcome (Diskhaler).

The respiratory system consists generally of three components: the tracheal/pharyngeal, the bronchial and the alveolar. It is known that particles of 10-50 microns migrate to the tracheal/pharyngeal component. Particles of about 5-10 microns migrate to the bronchial component, and particles of 0.5 to 5 microns migrate to the alveolar component. Particles less than 0.5 microns in size are not retained.

The mass median aerodynamic diameter (MMAD) is predictive of where in the lung a given particle will end up. The MMAD is usually expressed in microns. A related parameter is the geometric standard deviation (GSD). A GSD of 1 is equal to a normal distribution. A GSD of less than one indicates a narrow size dispersion and a GSD of more than 1 indicates a broad size dispersion.

The material can be transferred from the delivery device (e.g. via an aerosolization device) into the respiratory tract, down to the distal bronchi and alveoli, from where it can diffuse into the extracellular lung matrix. The delivery formulation should have physical characteristics which avoid clogging of the aerosol device and clumping of aerosolized particles. It should be noted that a viscous material, delivered slowly, may not cause clogging or plugging, whereas a less viscous material may, if delivered quickly.

Formulations of specific molecular weight, concentration, viscosity etc. are preferably produced by adding a volume of sterile delivery solvent (e.g., water or saline) to an amount of sterile, medical grade IL-7. Formulation temperatures of between about 0° to about 100° C, preferably between about 4° and 60° C and more preferably between about 15° and 37° C may be used in accordance with the present invention. Thus, the user can determine empirically the viscosity with a viscometer, and adjust the concentration accordingly to yield a viscosity adapted for delivery by the desired delivery mechanism (e.g., nebulizer, aerosolizer, inhaler etc.) to the selected target site in the

lungs. For delivery to the lungs at ambient temperature, the viscosity is preferably below about 1,000 cps, more preferably below about 100 cps, and most preferably below about 50 cps.

Another factor which should be considered in formulating an IL-7 solution for delivery to a selected target site in the respiratory tract is the droplet or particle size generated. This factor should be considered for aerosol as well as powder delivery pathways. Particle size is preferably below about 10 microns in diameter. More preferably, the particle size is between 2 and 5 microns. Raabe et al., reported a survey of particle size access to various airways in small laboratory animals using inhaled monodisperse aerosol particles. Raabe et al., *Ann. Occup. Hyg.* 1988, 32:53-63; incorporated herein by reference thereto. Similar analysis may be performed to inform the clinician as to the desirable particle size for delivery to a target site within the lung.

Particle size in accordance with a preferred mode of the present invention may be between about 2 microns and about 5 microns, thereby being adapted for delivery into the lung alveoli. Larger size particles are not as efficiently delivered through the distal bronchioles, whereas much smaller sizes tend to be exhaled before contacting the alveolar lining. Thus, whereas the therapeutic profile (e.g., duration, water retention, elastic recoil and matrix coverage) tend to increase with increasing molecular weight, the relative deliverability (i.e., frequency of particles within the 2-5 micron range) tends to decrease with increasing molecular weight.

In order to produce an aerosol which can be inhaled by human beings for distribution throughout the lung, the IL-7 can be aerosolized into appropriate droplet sizes as detailed above, preferably between about 2-5 microns in diameter. Some droplets larger than 5 microns in diameter may deposit in the nebulizer tubing or mask, mouth, pharynx, or laryngeal region. Droplets less than 2 microns in diameter tend not to be deposited in the respiratory tract, but are exhaled and lost. Droplet sizes of 2-5 microns can be achieved by selection of appropriate aerosol devices, solution concentration, compound molecular weight, and additives, in accordance with the teachings herein.

Additives such as surfactants, soaps, Vitamin E, and alcohol may be added to avoid clumping of droplets after they are produced, and to facilitate generation of small particles from an aerosol device. One embodiment of the invention includes IL-7 in combination with one or more of these additives.

A method of selecting breathable formulations for delivery to the lung by aerosol is to screen multiple formulations for those formulations which will produce droplets of less than 10 microns in diameter, more preferable less than 6 microns, most preferably 2-5 microns. Formulations which produce droplets larger than 10 microns are not suitable for delivery into the lung. Particle size distribution of the aerosolized mist for each formulation is measured with a device such as a Malvern Laser or a Cascade Impactor. This invention includes all molecular weight and concentration combinations of IL-7 that can be aerosolized into droplet sizes of under 10 microns, and more preferably between about 2-5 microns.

One embodiment of the invention involves use of an aerosol-generating device to produce an inhalable mist. One class of device to generate IL-7 aerosols is a spray atomizer. Another class of device to generate IL-7 aerosols is a nebulizer. Nebulizers are designed to produce droplets under 10 microns.

Many commonly used nebulizers may be used to aerosolize IL-7 for delivery to the lung: 1) compressed air nebulizers (examples of these include the AeroEclipse, Pari L.C., the Parijet and the Whisper Jet) and 2) ultrasonic nebulizers. Compressed air nebulizers generate droplets by shattering a liquid stream with fast moving air. One mode of the invention involves use of a compressed air nebulizer to aerosolize IL-7 solutions into droplets under 10 microns in size. Ultrasonic nebulizers use a piezoelectric transducer to transform electrical current into mechanical oscillations, which produces aerosol droplets from a liquid solution. Droplets produced by ultrasonic nebulizers are carried off by a flow of air. Another mode of the invention involves the use of an ultrasonic nebulizer to aerosolize IL-7 solutions into droplets less than 10 microns in size.

Another mode of this invention is use of a hand-held inhaler to generate IL-7 aerosols. This portable device will permit an individual to administer a single dose of mist, rather than a continuous "cloud" of mist into the patient's mouth. Individuals with bronchoconstrictive diseases such as asthma, allergies, or COPD often carry these hand-held inhalers (e.g., MDI and DPI) in their pocket or purse for use to alleviate a sudden attack of shortness of breath. These devices contain bronchodilator medication such as albuterol or atrovent. They would also be a convenient way to deliver IL-7 to patients.

For treatment via nebulizer, patients would inhale the aerosolized IL-7 solution via continuous nebulization, similar to the way patients with acute attacks of asthma or emphysema are treated with aerosolized bronchodilators. The aerosol may be delivered through tubing or a mask to the patient's mouth for inhalation into the lungs. Treatment time may last 30 minutes or less. The mouth is preferably used for inhalation (rather than the nose) to avoid "wasted" nasal deposition. To optimize the delivery rate of IL-7 via nebulizer, the volumetric flow rate (L/min) of the nebulizer preferably does not exceed two times the patient's minute ventilation, although this can be varied depending on the IL-7 formulation and the clinical status of the patient. This is because the average inspiratory rate is about twice the minute ventilation when exhalation and inhalation each represent about half of the breathing cycle. In one mode of the invention, a nebulizer with a volumetric flow rate of under 15 L/min is employed.

The particle size distribution generated from nebulizers is a function of a number of variables related to the nebulizer as well as the formulation (as discussed above). Nebulizer related factors for compressed air nebulizers include air pressure, air flow, and air jet diameter. Nebulizer related factors for ultrasonic nebulizers include ultrasound frequency, and rate/volume of air flow. In one mode of the invention, a compressed air nebulizer with specific air pressure, air flow, and hole diameter settings is used to generate droplets of a specific IL-7 formulation under 10 microns. In another mode, an ultrasonic nebulizer with specific frequency and hole diameter settings is employed to generate droplets of a specific IL-7 formulation under 10 microns.

Other considerations that determine selection of an ideal nebulizer and formulation include solution use rate (ml/min), aerosol mass output (mg/L), and nebulizer "hold up" (retained) volume (ml). The interaction among these factors will be appreciated by those of skill in the art.

Aerosolized IL-7 could be delivered from nebulizer to a patient's respiratory tract via face mask, nonrebreather, nasal cannula, nasal covering, "blow by" mask, endotracheal tube, and Ambu bag. All of these connections between the patient and nebulizer are considered to fall within the scope of the present invention.

In addition to delivery of IL-7 via nebulizer and via direct instillation through the anterior aspect of the trachea, IL-7 could also be delivered to target lung tissue via bronchoscopy. Bronchoscopy is a procedure where pulmonary physicians insert a scope into a patient's mouth, through the trachea, and into the bronchial airways. The scope allows visualization and access into the lungs for diagnosis (e.g. collection of bronchial alveolar lavage samples) and therapeutic procedures (e.g. placement of stents). One mode of the invention involves delivery of IL-7 via a bronchoscope to specific regions of the lung.

In addition to delivery via unassisted inhalation, another embodiment of the invention involves delivery of aerosolized IL-7 under positive pressure ventilation. A commonly used ventilatory assist device is CPAP: Continuous Positive Airway Pressure. In this application, a breathing mask is sealed around the mouth of a patient. The patient is then administered oxygen through the mask at a certain pressure to facilitate inspiration. Delivery of IL-7 through a CPAP mask might enhance delivery of material to the deep airways. To facilitate delivery to the alveoli and transfer across the alveolar epithelial barrier, the IL-7 could be delivered while the patient is being ventilated with positive end expiratory pressure (PEEP).

Another mode of the invention is to deliver aerosolized IL-7 with a device that delivers material when the patient generates a certain level of negative inspiratory pressure. Another mode of the invention is to deliver IL-7 in conjunction with ventilation through an endotracheal tube.

In another aspect of the invention, methods and formulations that include a IL-7 are disclosed for the delivery of drugs or other agents (e.g. imaging agents) to the lung for local or systemic therapies. The invention also includes methods and formulations to deliver IL-7 to the lung before or after delivery of a drug to enhance the efficacy of the drug, in an unaltered form as a depot for slow release of drugs, in unaltered form as a drug carrier, or in an altered form as a drug conjugate.

Effective dosages and schedules for administering the IL-7 polypeptides may be determined empirically (e.g. using the models disclosed herein), and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of IL-7 polypeptide that can be administered will vary depending on, for example, the mammal which will receive the IL-7 polypeptide, the route of administration, the particular type of molecule used (e.g. polypeptide, polynucleotide etc.) used and other drugs being administered to the mammal.

The IL-7 polypeptide may also be administered to the mammal in combination with effective amounts of one or more other therapeutic agents. The one or more other therapeutic agents or therapies may include, but are not limited to IFN-γ, anti–TGF-β antibodies, soluble TGF-β receptors and the TGF-β inhibitor decorin. The amounts of the therapeutic agent depend, for example, on what type of drugs are used, the pathological condition being treated, and the scheduling and routes of administration but would generally be less than if each were used individually.

Following administration of a IL-7 polypeptide to the mammal, the mammal's physiological condition can be monitored in various ways well known to the skilled practitioner. The therapeutic effects of the IL-7 polypeptides of the invention can be examined in *in vitro* assays and using *in vivo* animal models. A variety of well known animal models can be used to further understand the role of the IL-7 in the development and pathogenesis of IPF, and to test the efficacy of the candidate therapeutic agents.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration. That result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease or any other desired

alteration of a biological system. For example, in a further embodiment of the invention, there are provided articles of manufacture and kits containing materials useful for treating pathological conditions with IL-7. The article of manufacture comprises a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition having an active agent which is effective for treating pathological conditions such as IPF. The active agent in the composition is preferably IL-7. The label on the container indicates that the composition is used for treating pathological conditions with IL-7.

The kit of the invention comprises the container described above and a second container comprising a buffer. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

C. Illustrative Embodiments of the Invention

The invention disclosed herein has a number of embodiments. A preferred embodiment of the invention is a method of treating idiopathic pulmonary fibrosis in a mammalian subject by administering a therapeutically effective amount of IL-7 to the subject. Preferably the IL-7 is human IL-7 such as the IL-7 having the polypeptide sequence shown in SEQ ID NO: 1. In such embodiments the IL-7 can be administered by any one of methods known in the art, preferably by one of the methods typically used to administer agents to the lungs, for example, through the use of a nebulizer, atomizer, inhaler or the like. Most preferably the IL-7 is administered to the subject via a nebulizer.

Optionally, the IL-7 polypeptides used in the invention can be conjugated to a polyol such as polyethylene glycol. In addition, the IL-7 formulations of the invention typically comprise a pharmaceutically acceptable carrier. In preferred embodiments of the invention, the IL-7 formulations also include a liposome composition such as dimimyristol phosphatidyl choline (DMPC). Such liposome compositions are preferred

embodiments of the invention due to material characteristics such as being in a solidified form when refrigerated or nebulized at ambient room temperature but in a liquid form when at body temperature.

In another mode of administration, the formulation administered to the mammalian subject includes a vector encoding the IL-7 sequence shown in SEQ ID NO: 1, with the vector being administered so that the cells of the mammal are transduced by the vector and subsequently express IL-7 in an amount sufficient to, for example, inhibit Transforming Growth Factor-β (TGF-β, see, e.g., accession nos. AAA50405 and AAK56116) production or signaling in the appropriate physiological context (e.g. in the lung of an individual suffering from idiopathic pulmonary fibrosis).

Other embodiments of the invention include methods utilizing IL-7 and an additional agent to effect a physiological process in a pulmonary fibrosis fibroblast. A typical embodiment of such methods is a method of treating interstitial pulmonary fibrosis in a mammalian subject by administering a therapeutically effective amount of the IL-7 polypeptide shown in SEQ ID NO: 1 and an amount of interferon-γ (IFN-γ, see, e.g. accession nos. AAB59534 and P01580) sufficient to effect one of the biological processes identified herein as associated with IPF and therapeutic methods for treating IPF such as an increase in the expression of Smad7 in a pulmonary fibrosis fibroblast in the subject (see, e.g. Accession Nos. O35253 and O15105 and Nakao et al., Nature 389 (6651), 631-635 (1997) and Hayashi et al., Cell 89 (7), 1165-1173 (1997)). Such methods can include additional or alternative steps with other agents such as administering an amount of anti–TGF-β antibody, soluble TGF-β receptor, or decorin sufficient to inhibit TGF-β induced collagen synthesis in a pulmonary fibrosis fibroblast in the subject.

Related embodiments of the invention involve the administration of an amount of IL-7 sufficient to effect on one or more of the biological processes that are identified herein as being associated with TGF-β's role in pathological conditions such as idiopathic pulmonary fibrosis. As described below such methods can be used in cell based assays. Such methods can also be used in therapeutic contexts. One such embodiment is a method of treating idiopathic pulmonary fibrosis in a mammalian

subject comprising administering an amount of IL-7 having the sequence shown in SEQ ID NO: 1 to the subject, wherein the amount of IL-7 is sufficient to decrease TGF-β polypeptide production in a pulmonary fibrosis fibroblast in the subject. Another embodiment of the invention is a method of inhibiting TGF-β signaling in a pulmonary fibrosis fibroblast in a IFN-γ independent manner comprising exposing the pulmonary fibrosis fibroblast to an amount of IL-7 sufficient to sufficient to increase the expression of Smad7 in the pulmonary fibrosis fibroblast. Yet another related embodiment of the invention is a method of inhibiting the expression of TGF-β polypeptide in a pulmonary fibrosis fibroblast comprising exposing the pulmonary fibrosis fibroblast to an amount of IL-7 sufficient to inhibit the phosphorylation of Smad3 in the pulmonary fibrosis fibroblast (see, e.g. Accession No. Q92940 and Zhang et al., Nature 383 (6596), 168-172 (1996)). Yet another embodiment of the invention is a method of inhibiting TGF-β induced collagen synthesis in a pulmonary fibrosis fibroblast comprising exposing the pulmonary fibrosis fibroblast to an amount of IL-7 sufficient to inhibit the expression of TGF-β polypeptide in the pulmonary fibrosis fibroblast.

As disclosed herein, because IL-7 inhibits both the production of TGF-β in pulmonary fibrosis fibroblasts as well as TGF-β signaling in these cells and because TGF-β plays a pivotal role in tissue fibrosis including pulmonary fibrosis, the models disclosed herein (e.g. those described in Example 2 below) can be used in cell based assays designed to assess the effects of compounds which can modulate the production and or signaling of TGF-β in pulmonary fibrosis fibroblasts. For example the disclosure provided herein allows one to examine the effects that test compound has on the ability of IL-7 to modulate the production and or signaling of TGF-β and to identify compounds which modulate TGF-β production and or signaling (and or the resulting physiological effects of modulating TGF-β production and or signaling) in an advantageous manner.

Such cell based assays can also be used for example to evaluate how various IL-7 formulations impact specific physiological processes in pulmonary fibrosis fibroblasts

that are associated with a pathology such as idiopathic pulmonary fibrosis. Related embodiments of the invention are cell based assays which can be used to dissect specific physiological aspects of pathological conditions such as idiopathic pulmonary fibrosis. Related embodiments of the invention are cell based assays which can be used to evaluate how additional agents may effect such processes. One such embodiment of the invention is a method of identifying an agent capable of modulating a TGF-β associated process in a pulmonary fibrosis fibroblast by exposing the pulmonary fibrosis fibroblast to an amount of IL-7 and additionally exposing the pulmonary fibrosis fibroblast to a small molecule or polypeptide agent and observing the agent's effect on a TGF-β associated process such as the inhibition of TGF-β signaling. In an illustrative embodiment of the invention the agent is a polypeptide selected from the group consisting of interferon-γ, anti–TGF-β antibody and soluble TGF-β receptor.

The cell based assay methods described herein can be employed in a number of contexts. For example the methods described above can be practiced serially as the effects of compounds that have the ability modulate TGF-β production and or signaling is examined. In one such embodiment of the invention, TGF-β production and or signaling in response to IL-7 in a specific PFF model is first examined to determine the effects of IL-7 in that specific context. A variation of the method can then be repeated using a test compound in place of IL-7 and the effects on TGF-β production and or signaling in response to the test compound in the model then being examined to identify molecules which can produce physiological effects that are similar or dissimilar to IL-7. In a related embodiment IL-7 and a test compound can be added simultaneously to see if the test compound can modulate the effects of IL-7 in a manner that may have some clinical applicability, for example to modulate TGF-β production and or signaling with fewer side effects etc.

As these models measure and compare a number or related physiological processes including TGF-β production, TGF-β signaling, collagen synthesis and the progression of pulmonary fibrosis and because these phenomena are shown herein to be

linked, the models provide internal references which facilitates the identification new molecules of interest and the dissection their effects on cellular physiology. Moreover, these methods provide a particularly useful clinical model because they parallel methods of treatment. Specifically, treating IPF with IL-7 entails a method of effecting or modulating TGF-β production and or signaling in a PFF by exposing the cells to IL-7.

Any molecule known in the art can be tested for its ability to mimic or modulate (increase or decrease) the activity of IL-7 as detected by a change in the level of certain cytokines. For identifying a molecule that mimics or modulates IL-7 activity, candidate molecules can be directly provided to a cell or test subject in vivo or in vitro in order to detect the change in cytokine expression. Moreover, any lead activator or inhibitor structure known in the art can be used in conjunction with the screening and treatment methods of the invention. Such structures may be used, for example, to assist in the development of activators and/or inhibitors of IL-7.

This embodiment of the invention is well suited to screen chemical libraries for molecules which modulate, e.g., inhibit, antagonize, or agonize or mimic, the activity of IL-7 as measured by one of the phenomena disclosed herein. The chemical libraries can be peptide libraries, peptidomimetic libraries, chemically synthesized libraries, recombinant, e.g., phage display libraries, and in vitro translation-based libraries, other non-peptide synthetic organic libraries, etc.

Exemplary libraries are commercially available from several sources (ArQule, Tripos/PanLabs, ChemDesign, Pharmacopoeia). In some cases, these chemical libraries are generated using combinatorial strategies that encode the identity of each member of the library on a substrate to which the member compound is attached, thus allowing direct and immediate identification of a molecule that is an effective modulator.

Other embodiments of the invention include methods for the preparation of a medication for the treatment of pathological conditions including IPF by preparing a IL-7 composition for administration to a mammal having the pathological condition. A related method is the use of an effective amount of a IL-7 in the preparation of a medicament for the treatment of idiopathic pulmonary fibrosis. Such methods typically

involve the steps of including an amount of IL-7 sufficient to inhibit TGF-β production and/or TGF-β signaling in vivo and an appropriate amount of a physiologically acceptable carrier. As is known in the art, optionally other agents can be included in these preparations.

As shown for example in Figure 1, IL-7 decreases TGF-β production in PFF, but not in NF (while IFN-γ decreased TGF-β production in both NF and PFF). Consequently, the disclosure provided herein further provides methods for identifying pulmonary fibrosis fibroblasts based on their different responses to IL-7. One embodiment of the invention entails a method of identifying a NF and/or a PFF by exposing the fibroblast that is being examined to IL-7 and then observing the effects of IL-7 on TGF-β production, with those fibroblasts in which IL-7 decreases TGF-β production being identified as PFF. Such methods can be used for example in conjunction with other methods known in the art for identifying PFFs.

Throughout this application, various patents, patent applications, accession numbers (which, as is known in the art provide a reference of sequence and publication information), and other publications etc. are referenced (e.g. WO 00/38706). The disclosures of these publications etc. are hereby incorporated by reference herein in their entireties. The present invention is not to be limited in scope by the embodiments disclosed herein, which are intended as single illustrations of individual aspects of the invention, and any that are functionally equivalent are within the scope of the invention. Various modifications to the models and methods of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and teachings, and are similarly intended to fall within the scope of the invention. Such modifications or other embodiments can be practiced without departing from the true scope and spirit of the invention. However, the invention is only limited by the scope of the appended claims.

EXAMPLES

EXAMPLE 1: METHODS AND MATERIALS FOR EXAMINING IMMUNOMODULATORY MOLECULES SUCH AS IL-7 IN IDIOPATHIC PULMONARY FIBROSIS

Cell culture. Pulmonary fibrosis fibroblasts (PFFs) were isolated from lung resection specimens obtained from patients with IPF. Normal fibroblasts (NFs) were isolated from patients with nonfibrotic diseases. U4A, U3A, and U4A/JAK1 cell lines were generously provided by George R. Stark (Cleveland Clinic Foundation, Cleveland, Ohio, USA). The cells were maintained in 5% CO_2 in air as monolayers at 37°C in 75-cm^2 tissue culture flasks containing 20 ml of DMEM supplemented with 10% FBS, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and 2 mM glutamine (JRH Biosciences, Lenexa, Kansas, USA).

Cytokines and antibodies. Human activated recombinant TGF-β1 (3.2×10^4 units/μg), recombinant human IL-7 (10^5 units/μg), recombinant mouse IL-7 (5×10^4 units/μg), recombinant human IFN-γ (2×10^4 units/μg), and mouse anti-human IFN-γ monoclonal neutralizing antibody were obtained from R&D Systems Inc. (Minneapolis, Minnesota, USA). Anti-mouse IFN-γ monoclonal neutralizing antibody was purified by affinity chromatography from ascites of SCID mice, which were generated 3–4 weeks after intraperitoneal injection of 10^6 R4-462 hybridoma cells per mouse (American Type Culture Collection, Rockville, Maryland, USA) (see e.g. Spitalny, 1984 *J. Exp. Med.* **159**:1560–1565).

Dominant negative Smad7. The retroviral vector containing a full-length Smad7 cDNA was generously provided by Rik Derynk (University of San Francisco, San Francisco, California, USA). The Smad7 dominant negative mutant was constructed by a 25–amino acid deletion at the COOH terminus of Smad7. After transfection to the PT-67 retroviral packaging cell line by a liposomal-mediated method (Effectene method; QIAGEN Inc., Valencia, California, USA), the supernatant containing high titer of retrovirus expressing dominant negative Smad7 was collected to transduce PFF cells.

Mice. Pathogen-free female C57BL/6 mice (6–8 weeks old) and CB17 SCID Beige mice (6–8 weeks old) were purchased from the Charles River Laboratories, Inc. (Wilmington, Massachusetts, USA) and maintained in the West Los Angeles Veterans Affairs Animal Research Facility.

Bleomycin-induced pulmonary fibrosis model. To induce pulmonary fibrosis, mice were treated with 0.15 U bleomycin in 25 μl 0.9% normal saline (NS) or 0.9% NS alone by intratracheal administration. One day before bleomycin instillation, mice were started on treatment with 50 μg of recombinant IL-7 or vehicle control followed by administration five times per week for 2 weeks by intraperitoneal injection. Fourteen days following bleomycin exposure, mice were euthanized and both lungs were removed for the determination of hydroxyproline, collagen, TGF-β, and IFN-γcontent. Separate experiments assessed whether IL-7–mediated antifibrotic activities are IFN-γ independent. Two days before bleomycin instillation, treatment was started with 100 μg of anti–IFN-γ neutralizing antibody or control antibody five times a week for 2 weeks by intraperitoneal injection to neutralize endogenous and IL-7–induced IFN-γ. In our previous studies we found that this dose of anti– IFN-γ neutralizing antibody had the capacity to neutralize > 95% circulating IFN-γ in vivo.

TGF-β and IFN-γ ELISA. Mouse anti-human TGF-β or IFN-γ monoclonal capture antibody and biotinylated detecting antibody pairs were obtained from R&D Systems Inc. Monoclonal anti–TGF-β antibody measures TGF-β1 (see, e.g. accession no. P01137), 2 (see, e.g., accession no. M19154), and 3 (see, e.g. accession no. J03241). ELISA was performed based on a horseradish peroxidase method as described previously (see e.g. Dubinett et al., 1993 *J. Immunol.* **151**:6670–6680; Dubinett et al., 1995 *J. Natl. Cancer Inst.* **87**:593–597; and Miller et al., 1993 *Blood.* **82**:3686–3694). OD of each

sample after color development was determined with a microplate reader (Dynatech Laboratories, Chantilly, Virginia, USA) at 450 nm.

RT-PCR. The human Smad7 sense primer (5'-AGC ACA CCA GCT CGG GGT TGA T-3') (SEQ ID NO: 3) and antisense primer (5'- AAC GAT CTG CGC TCG TCC GGC G-3') (SEQ ID NO: 4) correspond to the coding regions 308–330 and 872–849 of the human Smad7 cDNA with amplified DNA size of 564 bp. The housekeeping gene GAPDH was used as control for semiquantification. High-quality total RNA from 10^7 cells each of NF or PFF samples with or without IL-7 treatment (100 ng/ml) for 24 hours was prepared by the TRIzol method (GIBCO BRL; Life Technologies Inc., Grand Island, New York, USA). RT-PCR was performed at 42°C for 1 hour followed by 35 cycles of amplification using a PTC-100 programmable thermal cycler (MJ Research Inc., Watertown, Massachusetts, USA).

Collagen assay. Collagen assay was performed using the Sircol collagen assay method (Accurate Chemical & Scientific Corp., Westbury, New York, USA). Briefly, 1,000 µl of Sircol dye reagent was added to 100 µl of test samples or collagen standards and incubated at 25°C for 30 minutes. After centrifugation at 5,000 *g* for 5 minutes, the supernatants were drained off and discarded. One thousand µl of 0.5N NaOH was added to the collagen bound dye pellet to release the bound dye into solution. The OD of each sample was determined with a microplate reader (Dynatech Laboratories) at 540 nm.

Hydroxyproline assay. Lungs were harvested on day 14 after bleomycin administration (see e.g. Keane, M.P., et al. 1999 *J. Immunol.* **163**:5686–5692; Keane, M.P., et al. 1999 *J. Immunol.* **162**:5511–5518). One-half milliliter of lung homogenate was digested in 1 ml of 6N HCl for 8 hours at 120°C. Five microliters of citrate/acetate buffer (5% citric acid, 7.24% sodium acetate, 3.4% sodium hydroxide, and 1.2% glacial acetic acid, pH 6.0) and 100 µl of chloramines-T solution (282 mg of chloramines-T, 2 ml of *n*-propanol, 2 ml of

H2O, and 16 ml of citrate/acetate buffer, pH 6.0) were added to 5 µl of sample and incubated for 20 minutes. Next, 100 µl of Ehrlich's solution (2.5 g of 4-(dimethylamino) benzaldehyde, 9.3 ml of *n*-propanol, 3.9 ml of 70% perchloric acid) was added to each sample and incubated for 15 minutes at 65°C. The OD was determined at 550 nm on a DU 640 spectrophotometer (Beckman Instruments Inc., Fullerton, California, USA). Hydroxyproline (Sigma Chemical Co., St. Louis, Missouri, USA) concentrations from 0–10 µg/ml were used to construct a standard curve.

Statistical analysis. All in vitro results are representative of at least three independent experiments performed in triplicate. In vivo experiments were performed with 10 mice per group, which yields a power of > 90% to detect any difference among the treatment groups ($\alpha = 0.05$). Significance of differences between experimental and control values was calculated using the Student's *t* test.

TABLE 1: Human and Murine IL-7 Sequences

P13232: Interleukin-7 precursor (IL-7) Homo Sapiens 177 AA

MFHVSFRYIFGLPPLILVLLPVASSDCDIEGKDGKQYESVLMVSIDQLLDSMKEI GSNCLNNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKV SEGTTILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLL QEIKTCWNKILMGTKEH (SEQ ID NO: 1)

NP_032397: Interleukin-7 precursor (IL-7) Mus musculus 154 AA

MFHVSFRYIFGIPPLILVLLPVTSSECHIKDKEGKAYESVLMISIDELDKMTGTDS NCPNNEPNFFRKHVCDDTKEAAFLNRAARKLKQFLKMNISEEFNVHLLTVSQ GTQTLVNCTSKEEKNVKEQKKNDACFLKRLLREIKTCWNKILKGSI (SEQ ID NO: 2)

EXAMPLE 2: IL-7 DECREASES TGF-β PRODUCTION BY PFFS.

The disclosure presented herein demonstrates that IL-7 has the capacity to decrease fibroblast TGF-β production. In human primary cultures of pulmonary fibroblasts isolated from patients with IPF (see e.g. Keerthisingam, C.B., et al. 2001 *Am. J. Pathol.*

158:1411–1422), we found that IL-7 (100 ng/ml) has the capacity to downregulate the production of TGF-β which was quantified by ELISA using mAb's recognizing TGF-β, 2, and 3 (Figure 1a). IL-7, in contrast, does not alter TGF-β production in the primary cultures of normal fibroblasts derived from lung resection specimens from patients who do not have IPF (Figure 1b). Both PFFs and NFs express the IL-7 receptor (IL-7R). No difference was detected in the level of PFF and NF IL-7R expression when assessed by flow cytometry. Because IFN-γ has previously been shown to decrease TGF-β production, we compared the capacities of IFN-γ and IL-7 to downregulate TGF-β and sought to determine whether their effects were additive. Both PFF and NF were tested and were devoid of IFN-γ in the culture supernatant. We exposed PFF and NF to IL-7, IFN-γ 500 units/ml), IL-7 plus IFN-γ or IL-7 plus anti–IFN-γ monoclonal neutralizing antibody (5 μg/ml) (Figure 1, c and d). Whereas NF responded only to IFN-γ by downregulating TGF-β production, PFF showed decreased TGF-β following exposure to either IL-7 or IFN-γ. As predicted, the addition of anti– IFN-γ antibody had no effect on IL-7–mediated downregulation of TGF-β because fibroblasts did not produce IFN-γ. The results provide evidence that IL-7 is signaling downregulation of TGF-β in an IFN-γ–independent manner and that PFF may have an accessory IL-7 signaling pathway or a different regulatory mechanism that is not operative in NF.

EXAMPLE 3: IL-7R SIGNALING REQUIRES AN INTACT JAK1/STAT1 SIGNALING PATHWAY.

Activation of the JAK/STAT pathway has been implicated in IL-7R signaling. Accordingly, we tested whether the capacity of IL-7 to decrease TGF-β production was mediated through JAK/STAT signaling. Using JAK1-deficient (U4A) and STAT1-deficient (U3A) mutant fibroblast cell lines, we observed that IL-7 had no effect on TGF-β production (Figure 2). However, when an intact JAK/STAT signaling pathway was reconstituted by complementing U4A cells with JAK1 expression (U4A/JAK1), the

fibroblasts responded to IL-7 by downregulating TGF-β production in a dose-dependent manner (Figure 2). These findings indicate that the downregulation of TGF-β production in fibroblasts by IL-7 requires an intact JAK1/STAT1 signal transduction pathway. In contrast to fibroblasts from nonfibrotic lungs, the U4A/JAK1 fibroblast cell line shows decreased TGF-β in response to IL-7 and, in this regard, is similar to PFFs.

EXAMPLE 4: IL-7 DECREASES TGF-β-INDUCED COLLAGEN SYNTHESIS.

In addition to decreasing TGF-β production, another important contribution for a cytokine used therapeutically to modulate the fibrotic response would be the capacity to inhibit the effects of TGF-β signaling (see e.g. Ulloa, L., Doody, J., and Massague, J. 1999 *Nature.* **397**:710–713; Massague, J. 1998 *Annu. Rev. Biochem.* **67**:753–791). TGF-β is known to stimulate both fibroblast proliferation (see e.g. Border et al., 1994 *N. Engl. J. Med.* **331**:1286–1292; Broekelmann et al., 1991 *Proc. Natl. Acad. Sci. USA.* **88**:6642–6646) and collagen deposition (see e.g. Khalil et al.,1989 *J. Exp. Med.* **170**:727–737). To test whether IL-7 could modulate TGF-β induced collagen synthesis, we exposed PFF and NF to activated recombinant TGF-β1 alone (10 ng/ml) or TGF-β plus IL-7 (100 ng/ml). When used at this concentration, TGF-β did not significantly impact fibroblast proliferation at 24 hours in either NF or PFF. When stimulated with TGF-β PFF but not NF showed enhanced collagen production (Figure 3). Following preincubation in IL-7 (100 ng/ml) for 2 hours before the addition of TGF-β1, there was a marked reduction in the capacity for TGF-β to elicit fibroblast collagen synthesis in PFF (Figure 3). Interestingly, IL-7 had no effect on the constitutive level of collagen synthesis. This provides evidence that IL-7 inhibits collagen synthesis by blocking TGF-β signaling in PFF.

EXAMPLE 5: IL-7 INHIBITS TGF-β SIGNALING THROUGH THE INDUCTION OF SMAD7.

TGF-β signaling and regulation are mediated by a family of SMAD proteins (see e.g. Ulloa, L., Doody, J., and Massague, J. 1999 *Nature.* **397**:710–713; Massague, J. 1998 *Annu. Rev. Biochem.* **67**:753–791). Among the SMAD family, Smad7 is the major inhibitor of TGF-β signaling. Previous studies indicate that IFN-γ has the capacity to regulate Smad7 through the JAK/STAT pathway (see e.g. Ulloa, L., Doody, J., and Massague, J. 1999 *Nature.* **397**:710–713). Based on our observation that IL-7 also mediates its effects in PFF through the JAK/STAT pathway, we hypothesized that IL-7 interfered with TGF-β signaling and, therefore, decreased TGF-β induced collagen synthesis by enhancing Smad7 expression in PFF. As shown in Figure 4a, IL-7 increased Smad7 in pulmonary fibroblasts. In order to determine whether the IL-7–induced increase in Smad7 expression was the cause of IL-7–mediated inhibition of collagen synthesis, PFF were transduced with a Smad7 dominant negative construct. The levels of collagen synthesis in response to IL-7 were evaluated in both untransduced fibroblasts and the Smad7 dominant negative–transduced (Smad7DNtransduced) cells. In the presence of IL-7, Smad7DNtransduced fibroblasts maintained high-level TGF-β induced collagen synthesis, indicating that an IL-7–mediated increase in Smad7 led to inhibition of TGF-β signaling (Figure 4b).

EXAMPLE 6: IL-7 INHIBITS BLEOMYCIN-INDUCED PULMONARY FIBROSIS IN VIVO.

Based on the capacity of IL-7 to downregulate TGF-β production and block TGF-β signaling in human PFF, we tested whether recombinant IL-7 would limit the development of bleomycin-induced pulmonary fibrosis in vivo. Consistent with our in vitro findings, recombinant IL-7 administration (5 μg/mouse five times per week for 2 weeks) by intraperitoneal injection decreased bleomycin-induced pulmonary fibrosis, as indicated by a decrease in hydroxyproline and collagen content in mouse lung homogenates (Figure 5, a and b). IL-7 administration also decreased TGF-β production

but increased IFN-γ secretion in lung homogenates from mice with bleomycin-induced pulmonary fibrosis (Figure 5, c and d). These results indicated that IL-7 has in vivo antifibrotic activities. Because IL-7–treated lungs in the bleomycin-induced pulmonary fibrosis murine models had higher levels of IFN-γ we speculated that the IL-7–mediated antifibrotic response may depend, in part, on the overproduction of IFN-γ. In preliminary studies, we have determined that 1 μg of the purified anti-mouse IFN-γ monoclonal neutralizing antibody used in these studies was able to neutralize 400–800 ng of mouse IFN-γ in vitro. Administration of anti–IFN-γ neutralizing antibody intraperitoneally at a concentration of 100 μg per injection per mouse five times a week for 2 weeks had the capacity to neutralize more than 95% of circulating IFN-γ in vivo. To determine the importance of IFN-γ in the IL-7–mediated antifibrotic activities in vivo, we repeated the in vivo experiment (Figure 5) using anti– IFN-γ neutralizing antibody (100 μg/mouse five times per week for 2 weeks) administered by intraperitoneal injection and assessed hydroxyproline and collagen levels. The results indicated that IL-7 decreased bleomycin-induced pulmonary fibrosis in an IFN-γ independent manner (Figure 6).

WHAT IS CLAIMED IS:

1. A method of treating idiopathic pulmonary fibrosis in a mammalian subject comprising administering a therapeutically effective amount of IL-7 polypeptide to the subject.

2. A method as in claim 1, wherein the IL-7 polypeptide comprises the amino acid sequence shown in SEQ ID NO: 1.

3. A method as in claim 2, wherein the IL-7 is conjugated to a polyol.

4. A method as in claim 2, wherein the IL-7 polypeptide is administered to the lungs via a nebulizer, atomizer or inhaler.

5. A method as in claim 1, wherein the IL-7 is in a formulation comprising liposomes and a pharmaceutically acceptable carrier.

6. A method as in claim 1, further comprising the step of administering an amount of interferon-γ sufficient to increase the expression of Smad7 in a pulmonary fibrosis fibroblast in the subject.

7. A method as in claim 1, further comprising the step of administering an amount of anti–TGF-β antibody, soluble TGF-β receptor, or decorin sufficient to inhibit TGF-β induced collagen synthesis in a pulmonary fibrosis fibroblast in the subject.

8. A method of treating idiopathic pulmonary fibrosis in a mammalian subject comprising administering an amount of IL-7 having the amino acid sequence shown in

SEQ ID NO: 1 to the subject, wherein the amount of IL-7 is sufficient to decrease TGF-β polypeptide production in a pulmonary fibrosis fibroblast in the subject.

9. A method as in claim 8, wherein the IL-7 is administered to the subject via a nebulizer.

10. A method as in claim 8, wherein the IL-7 is in a formulation comprising liposomes and a pharmaceutically acceptable carrier.

11. A method as in claim 8, further comprising the step of administering an amount of interferon-γ, anti–TGF-β antibody, soluble TGF-β receptor, or decorin sufficient to inhibit TGF-β induced collagen synthesis in a pulmonary fibrosis fibroblast in the subject.

12. A method of inhibiting TGF-β induced collagen synthesis in a pulmonary fibrosis fibroblast comprising exposing the pulmonary fibrosis fibroblast to an amount of IL-7 polypeptide having the amino acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 2, wherein the amount of IL-7 is sufficient to inhibit the expression of TGF-β polypeptide in the pulmonary fibrosis fibroblast.

13. A method as in claim 12, wherein the IL-7 is conjugated to a polyol.

14. A method as in claim 12, wherein the IL-7 has the polypeptide sequence shown in SEQ ID NO: 1.

15. A method as in claim 12, wherein the method further includes exposing the pulmonary fibrosis fibroblast to a small molecule or polypeptide agent and observing the agent's effect on the inhibition of TGF-β induced collagen synthesis in the pulmonary fibrosis fibroblast.

16. A method as in claim 15, wherein the agent is a polypeptide selected from the group consisting of interferon-γ, anti–TGF-β antibody, soluble TGF-β receptor.

17. A method of inhibiting TGF-β signaling in a pulmonary fibrosis fibroblast in a IFN-γ independent manner comprising exposing the pulmonary fibrosis fibroblast to an amount of IL-7 polypeptide having the amino acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 2, wherein the amount of IL-7 is sufficient to increase the expression of Smad7 in the pulmonary fibrosis fibroblast.

18. A method as in claim 17, wherein the IL-7 has the polypeptide sequence shown in SEQ ID NO: 1.

19. A method as in claim 17, wherein the method further includes exposing the pulmonary fibrosis fibroblast to a small molecule or polypeptide agent and observing the agent's effect on the inhibition of TGF-β signaling.

20. A method as in claim 18, wherein the pulmonary fibrosis fibroblast is exposed to an IL-7 polypeptide expressed by a mammalian cell that has been transduced with an expression vector encoding the IL-7 polypeptide, wherein the expression vector has been administered to a mammalian subject.

21. A method of treating idiopathic pulmonary fibrosis in a mammalian subject comprising administering a therapeutically effective amount of a polypeptide capable of inhibiting TGF-β production or TGF-β signaling in a interferon-γ independent manner.

22. Use of an effective amount of an IL-7 in the preparation of a medicament for the treatment of idiopathic pulmonary fibrosis.

23. The use of an effective amount of IL-7 according to claim 22, wherein the IL-7 comprises a polypeptide having the amino acid sequence shown in SEQ ID NO: 1

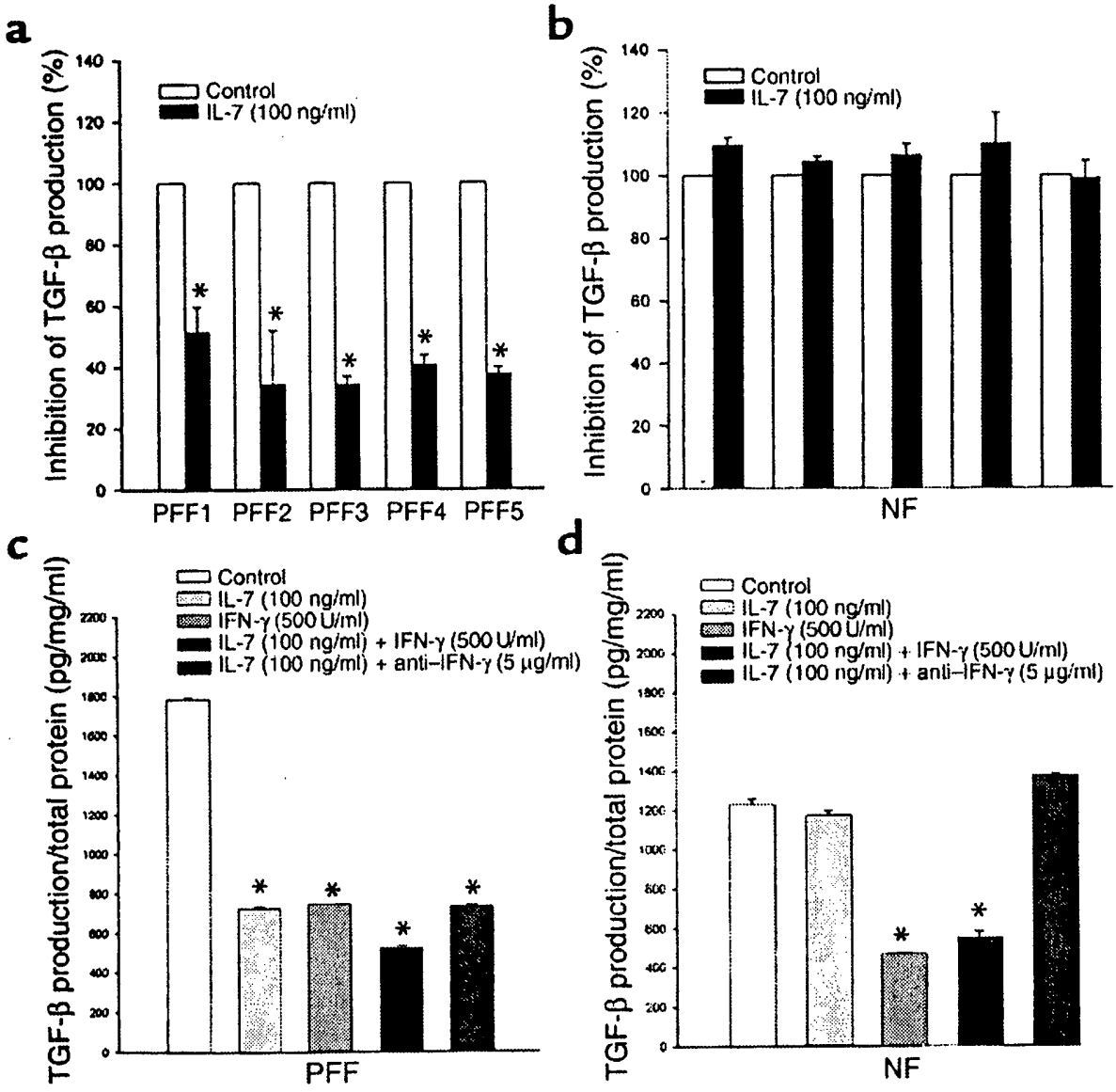
a
Inhibition of TGF-β production (%)
Control
IL-7 (100 ng/ml)
PFF1 PFF2 PFF3 PFF4 PFF5
b
Inhibition of TGF-β production (%)
Control
IL-7 (100 ng/ml)
NF
c
TGF-β production/total protein (pg/mg/ml)
Control
IL-7 (100 ng/ml)
IFN-γ (500 U/ml)
IL-7 (100 ng/ml) + IFN-γ (500 U/ml)
IL-7 (100 ng/ml) + anti-IFN-γ (5 μg/ml)
PFF
d
TGF-β production/total protein (pg/mg/ml)
Control
IL-7 (100 ng/ml)
IFN-γ (500 U/ml)
IL-7 (100 ng/ml) + IFN-γ (500 U/ml)
IL-7 (100 ng/ml) + anti-IFN-γ (5 μg/ml)
NF

Figure 1a - 1d

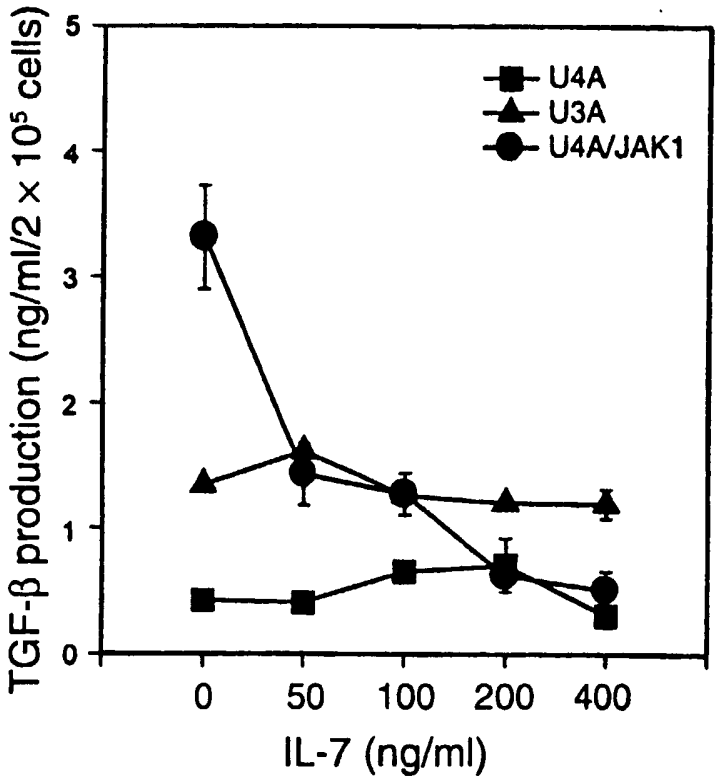
TGF-β production (ng/ml/2 × 10⁵ cells)
0
1
2
3
4
5
U4A
U3A
U4A/JAK1
0
50
100
200
400
IL-7 (ng/ml)

Figure 2

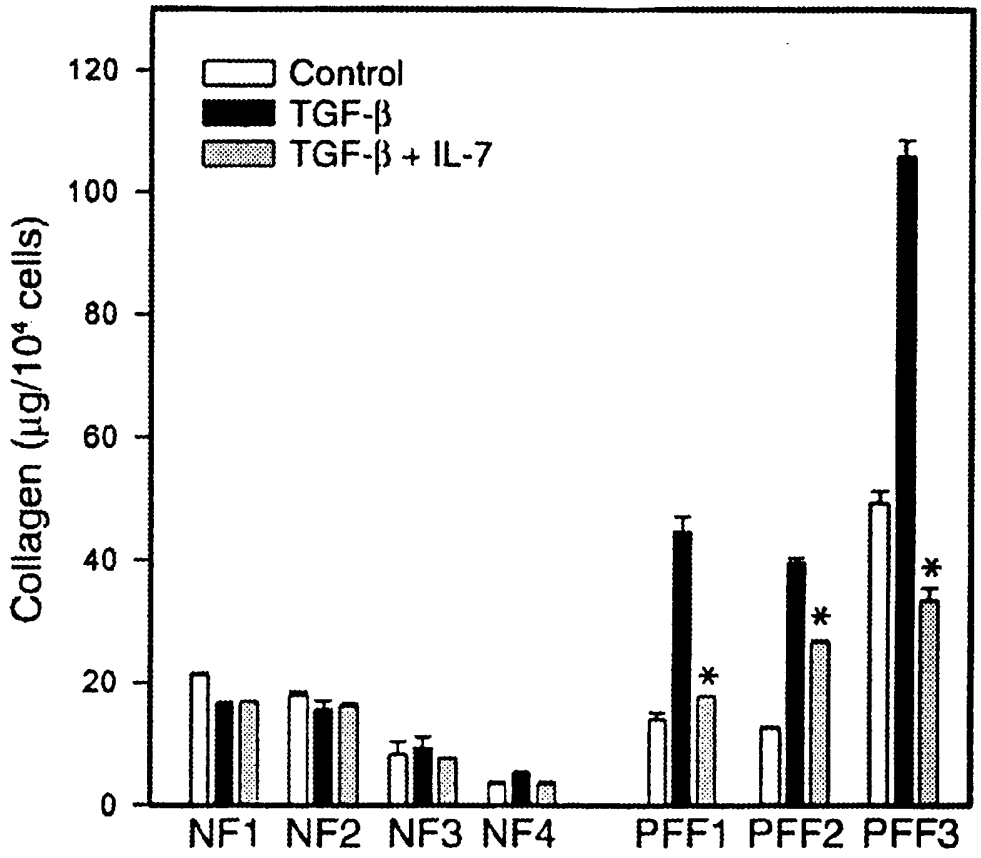
Control
TGF-β
TGF-β + IL-7
Collagen (μg/10⁴ cells)
120
100
80
60
40
20
0
NF1
NF2
NF3
NF4
PFF1
PFF2
PFF3
*
*
*

Figure 3

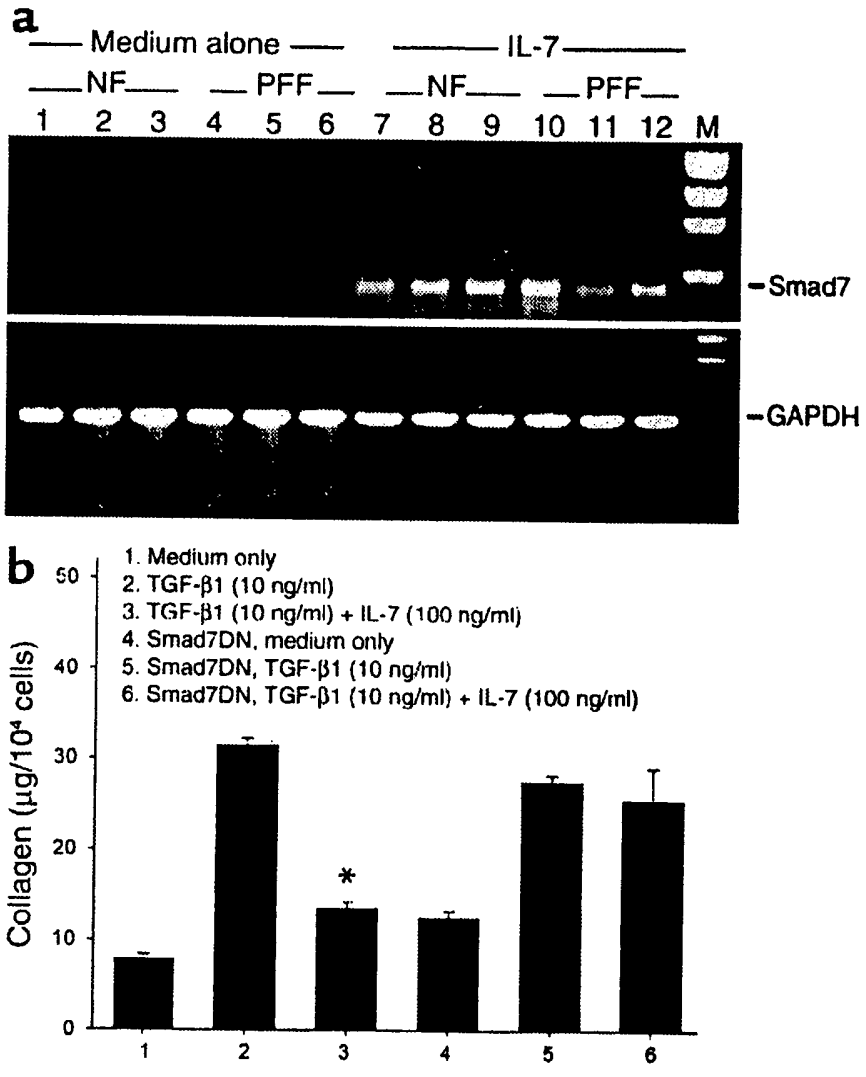
a
Medium alone
IL-7
NF
PFF
NF
PFF
1 2 3 4 5 6 7 8 9 10 11 12 M
Smad7
GAPDH
b
1. Medium only
2. TGF-β1 (10 ng/ml)
3. TGF-β1 (10 ng/ml) + IL-7 (100 ng/ml)
4. Smad7DN, medium only
5. Smad7DN, TGF-β1 (10 ng/ml)
6. Smad7DN, TGF-β1 (10 ng/ml) + IL-7 (100 ng/ml)
Collagen (μg/10⁴ cells)
0
10
20
30
40
50
*
1 2 3 4 5 6

Figure 4a-4b

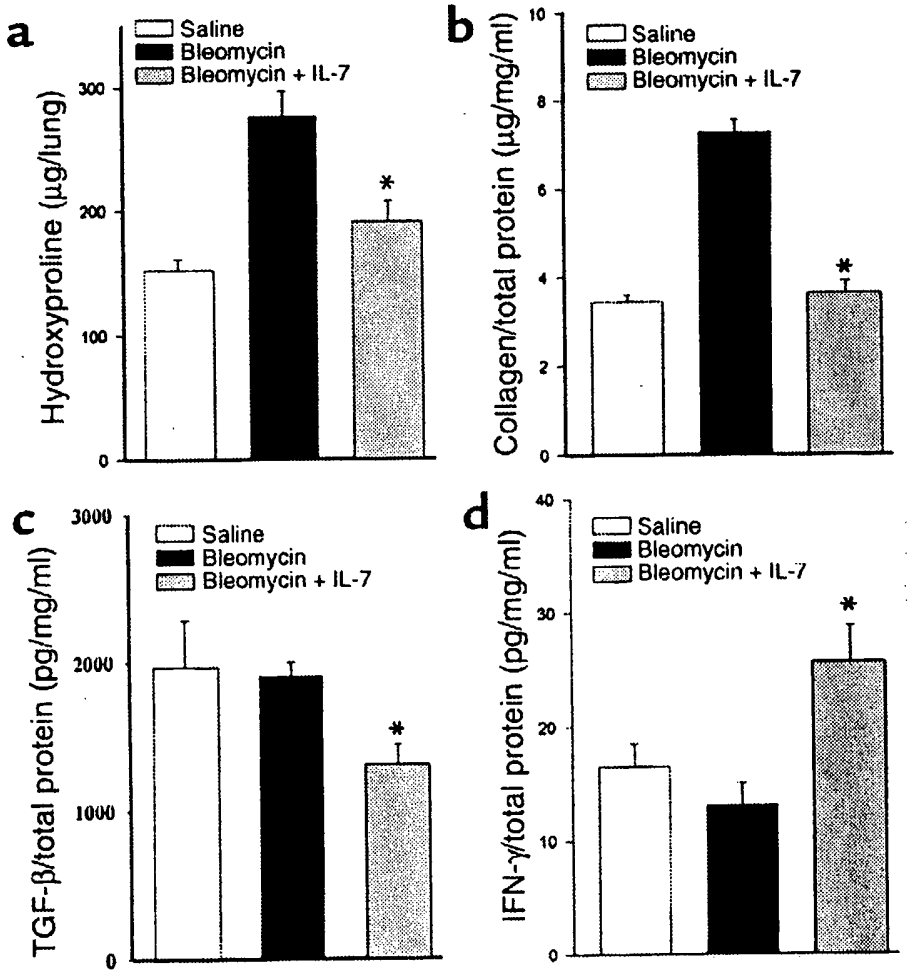
a
Saline
Bleomycin
Bleomycin + IL-7
Hydroxyproline (µg/lung)
0
100
200
300
*
b
Saline
Bleomycin
Bleomycin + IL-7
Collagen/total protein (µg/mg/ml)
0
2
4
6
8
10
*
c
Saline
Bleomycin
Bleomycin + IL-7
TGF-β/total protein (pg/mg/ml)
0
1000
2000
3000
*
d
Saline
Bleomycin
Bleomycin + IL-7
IFN-γ/total protein (pg/mg/ml)
0
10
20
30
40
*

Figure 5a – 5d

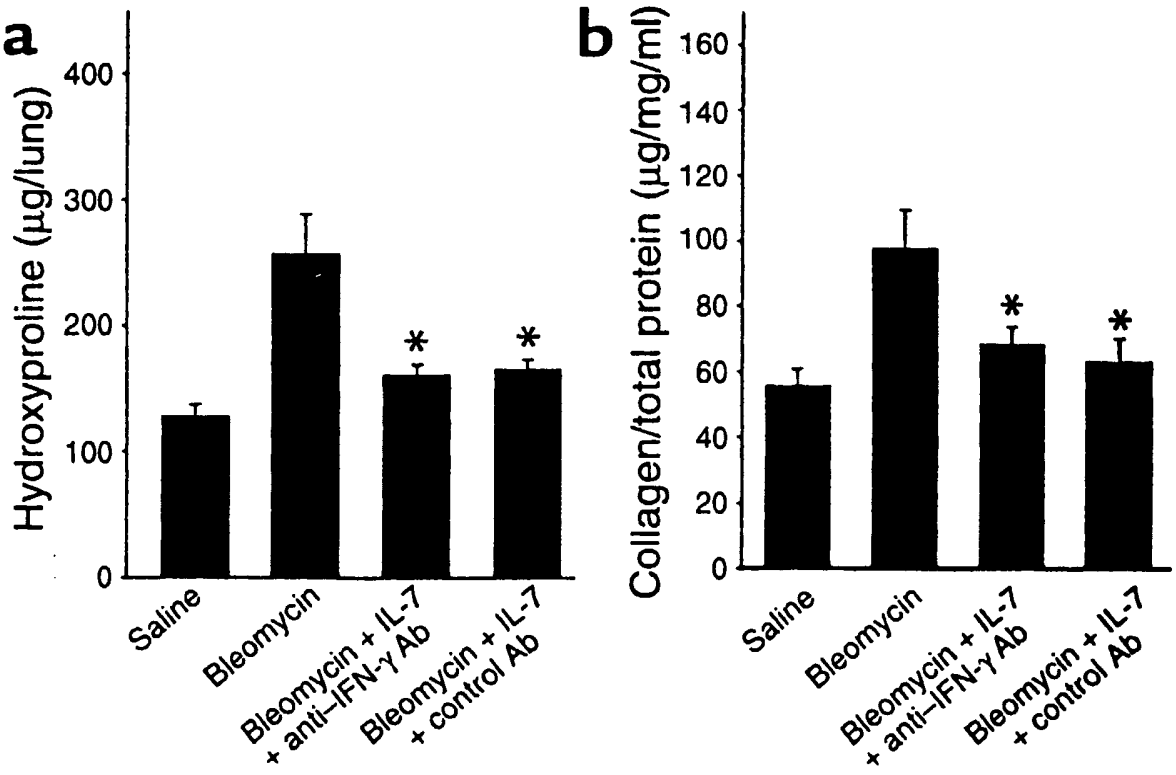
a
Hydroxyproline (µg/lung)
400
300
200
100
0
Saline
Bleomycin
Bleomycin + IL-7 + anti-IFN-γ Ab
Bleomycin + IL-7 + control Ab
b
Collagen/total protein (µg/mg/ml)
160
140
120
100
80
60
40
20
0
Saline
Bleomycin
Bleomycin + IL-7 + anti-IFN-γ Ab
Bleomycin + IL-7 + control Ab

Figure 6a–6b

SEQUENCE LISTING

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Min Huang
Sherven Sharma
Raj K. Batra
Robert M. Strieter
Steven M. Dubinett

<120> METHODS OF USING INTERLEUKIN-7 TO MODULATE PHYSIOLOGICAL PROCESSES IN MAMMALIAN PULMONARY FIBROBLASTS

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        35                  40                  45
Leu Asp Ser Met Lys Glu Ile Gly Ser Asn Cys Leu Asn Asn Glu Phe
    50                  55                  60
Asn Phe Phe Lys Arg His Ile Cys Asp Ala Asn Lys Glu Gly Met Phe
65                  70                  75                  80
Leu Phe Arg Ala Ala Arg Lys Leu Arg Gln Phe Leu Lys Met Asn Ser
                85                  90                  95
Thr Gly Asp Phe Asp Leu His Leu Leu Lys Val Ser Glu Gly Thr Thr
            100                 105                 110
Ile Leu Leu Asn Cys Thr Gly Gln Val Lys Gly Arg Lys Pro Ala Ala
        115                 120                 125
Leu Gly Glu Ala Gln Pro Thr Lys Ser Leu Glu Glu Asn Lys Ser Leu
    130                 135                 140
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35 40 45
Asp Lys Met Thr Gly Thr Asp Ser Asn Cys Pro Asn Asn Glu Pro Asn
50 55 60
Phe Phe Arg Lys His Val Cys Asp Asp Thr Lys Glu Ala Ala Phe Leu
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Asn Arg Ala Ala Arg Lys Leu Lys Gln Phe Leu Lys Met Asn Ile Ser
85 90 95
Glu Glu Phe Asn Val His Leu Leu Thr Val Ser Gln Gly Thr Gln Thr
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