Gamma Interferon Induced Organ Regeneration

Richard J. Sharpe, M.D.

Email: rjsharmd@gmail.com

Published online: October 12, 2008

ABSTRACT

The salamander is the only vertebrate that has a substantial capacity to regenerate severed limbs and damaged organs. A necessary condition for this response is the lack of scar formation (fibrosis) at the site of injury. I postulate that suprapharmacologic doses of gamma interferon administered locally to the site of traumatic injury or disease induced organ capacity loss might unlock regenerative capabilities in humans. Preliminary data involving a case of advanced pulmonary fibrosis (scarring) is presented wherein suprapharmacologic doses of inhaled gamma interferon induced the regeneration of functional lung parenchyma. Moreover, I postulate that potent inhibition of fibrosis in the adult human may allow the activation of embryonic development programs at the site of organ or tissue injury.

INTRODUCTION

Injury in the adult human is typically followed by the healing response which includes the laying down of a disordered collagen matrix [1-4]. This scar or fibrotic response has been postulated to be one of the impediments to regeneration of the damaged organ [1-4].

The adult salamander is the most well studied model of vertebrate organ and tissue regeneration in the adult [1-4]. An adult salamander can grow exact replacements for severed limbs and other lost body parts.

The early stages of tissue injury in the salamander and the adult human are similar. However, while a scar forms in humans at the site of tissue or organ injury, scarring does not occur in the salamander. Instead, the salamander reactivates an embryonic development program to repair or replace the large body part (tissue or organ) [1-4].

Gamma interferon has been postulated to be a useful inhibitor of fibrosis [5]. However, to date gamma interferon has not shown activity in human fibrotic disease. In fact, in one study where gamma interferon was administered subcutaneously, it failed in a trial of pulmonary fibrosis [6]. I postulate this failure was due to the lack of local suprapharmacologic dosing of gamma interferon.

THE HYPOTHESIS

Local delivery of suprapharmacologic doses of gamma interferon to the site of injury to a human organ or tissue prevents scarring (fibrosis) allowing tissue or organ regeneration in the adult. Additionally, long-term delivery of suprapharmacologic doses of gamma interferon may unlock embryonic tissue regeneration programs even after substantial scar formation (fibrosis) has occurred.

EVALUATION OF THE HYPOTHESIS

Fibrosis inhibition is a necessary condition for limb and organ regeneration in the adult [1-4]. Suprapharmacologic doses of gamma interferon were effective in an end-stage patient with pulmonary fibrosis (severe scarring of the lung parenchyma) (see preliminary data).

Traumatic injury or injury due to disease or therapeutic interventions can result in loss of organ capacity, loss of function of limbs and unsightly scars. By administering high dose gamma interferon (on the order of 20 million units per day or more) to the site of trauma or disease, fibrosis may be suppressed to a degree that allows regeneration of the organ or tissue. Delivery of gamma interferon can be intermittent or continuous via inhalation, injection, implantable polymers or pumps.

This approach is readily testable in animal models and humans.

PRELIMINARY DATA – REPORT OF A CASE

Pulmonary fibrosis (lung scarring) is thought to be caused by many factors ranging from toxic insults to the lung as is seen with chemotherapeutic agents such as bleomycin, as well as radiation therapy for breast, lung, lymphoma and other cancers. Inhalation of toxic or cell mediated immunity triggering allergens can also cause a fibrotic response to lung injury. An autoimmune disease targeting primarily lung tissue or an autoimmune disease associated with other autoimmune diseases, such as systemic scleroderma or rheumatoid arthritis with associated lung involvement is the basis of many cases of interstitial pulmonary fibrosis [1].

The usual interstitial pneumonitis variant (UIP) of interstitial pulmonary fibrosis probably represents the end stages of a more acute form of the disease [7]. The UIP form is characterized histologically by replacement of lung parenchyma by substantial amounts of collagen (scar tissue) in the interstitium and a chronic mononuclear cell inflammatory infiltrate with a relative absence of eosinophils [7,8].

Typically the fibrotic process impairs lung elasticity, reduces total lung capacity (TLC) reduces forced vital capacity (FVC), reduces the forced expiratory volume in one second (FEV1), reduces alveolar volume (Va) and substantially impairs gas exchange (Dsb_{adj}). The gold standard for diagnosis is an open lung biopsy. High resolution CT scan typically shows “ground glass” changes which can provide a high degree of certainty in making the diagnosis [5-13].

Many of these patients, including the patient described in this report, complain of a gradual increase in a dry cough over months or years and a progressive increase in dyspnea on exertion over the same time period [7].

The prognosis of interstitial fibrosis of all causes is poor, with life expectancy of 2-6 years [7,9] and the average life expectancy at the time of diagnosis being 2.8 years [5-13]. Patients with advanced disease (i.e., carbon monoxide diffusing capacity, Dsb_{adj}, loss of 60% or greater and substantial loss of forced vital capacity and lung volume) and advanced age (over 65) have a much worse prognosis [7,9]. Overall, less than 10% of patients with all types of interstitial lung fibrosis respond to standard therapy of systemic corticosteroids and immunosuppressants and/or chemotherapeutic agents such as cyclophosphamide or azathioprine or other immunosuppressive regimes [7].

Additionally, these treatments substantially increase the risk of pulmonary infection, which can be rapidly fatal in patients with limited pulmonary reserve. None of these treatments has been shown in well-designed clinical trials to substantially restore lost lung function or to prolong life and it is questionable whether they have a favorable impact on morbidity or life expectancy.

Recently, gamma interferon-1b, given subcutaneously at what the medical community currently considers high daily doses (200 micrograms per day, 4 million International Units per day) three days per week *has not* shown any substantial activity in the advanced phase of this disease [6].

Gamma interferon has been shown to increase host defense against bacteria, mycobacteria and other pulmonary pathogens including Staphylococcus aureus, Listeria, mycobacterium tuberculosis, mycobacterium leprae, atypical mycobacteria and Pneumocystis carinii. The mechanism of action may be its ability to increase superoxide production and nitric oxide production by immune cells, induction of 2° cytokines and increased antibody dependant cellular toxicity (ADCC) [10].

No therapy has been shown in a well-designed clinical trial to prolong life in any form of interstitial pulmonary fibrosis. In fact, therapeutic options are so poor, that many clinicians recommend only supportive care, particularly for elderly patients, due to the poor risk benefit ratio of current treatment [7]. If patients with interstitial pulmonary fibrosis are young enough and otherwise healthy, they many be considered for lung transplantation [11]. However, this procedure is very costly, there are not enough lungs to satisfy the need, and the survival benefit is moderate to poor. There is a great need for safe, highly effective and well-tolerated treatments for pulmonary fibrosis of all causes and subtypes.

The direct infusion of an immune enhancer into the lungs of individuals suffering from interstitial pneumonitis in general, and autoimmune interstitial pneumonitis in particular, brings with it the risk of worsening the lung damage from increased inflammation, worsened autoimmunity, cellular damage by inflammatory cells and their products, as well as increased fibrosis as is seen with tumor necrosis factor alpha (TNF-α) [12].

Additionally, a capillary leak syndrome or acute respiratory distress syndrome (ARDS) is a possibility as is seen with TNF-α and interleukin-2 [13,14], even when these cytokines are administered systemically. Additionally, many cytokines induce secondary cytokines or chemokines which can cause further toxicity, especially locally.

One would expect *a priori*, that inhalation of high doses of gamma interferon might accelerate the pathological changes of interstitial pulmonary fibrosis both directly and indirectly through induction of 2° cytokines, or chemokines, induction of an ARDS response [13,14], direct damage to lung parenchyma through the release of highly reactive and damaging superoxide and nitric oxide species and a variety of other mechanisms.

I report a case of advanced UIP, which was put into complete remission, with near complete restoration of his lost lung function, which can only be explained by regeneration of new lung parenchyma. This response occurred in less than fourteen months with very little morbidity or toxicity with a novel two-agent regime, which included aerosolized, nebulized gamma interferon-1b starting at low doses and escalating to suprapharmacologic doses, and high dose intravenous immunoglobulin, which was added to control gamma interferon side effects.

History

A 79 year-old Caucasian male presented with a several year history of worsening dry cough and dyspnea on exertion. His environmental exposure history was remarkable for work in a university laboratory overseeing graduate students performing experiments on anti-oxidants and food biochemistry. During this work, he was exposed to numerous organic solvents and chemicals.

When he was in his late teens, he was in the Marine Corp and served five years on the front lines in World War II. He spent many weeks in cramped quarters aboard naval vessels in which asbestos was commonly used as insulation and as a fire retardant.

He smoked about one pack of cigarettes per day from his teens until quitting around the age of 52 (about 38 pack years). He drinks alcohol moderately.

Past Medical History

Unremarkable.

Family History

His father and mother both died at the age of 88 of “old age”. He has a daughter who has at least two autoimmune diseases; autoimmune thyroiditis and Sjögren’s syndrome.

Review of Systems

No known medication allergies; denies fever, chills, intolerance to heat or cold, ENT symptoms were unremarkable except he does require a hearing aid; denies chest pain, or extra heart beats; he denies musculoskeletal pain, except back and knee pain diagnosed as osteoarthritis; he denies headaches, depression, and difficulty in clarity of thinking. His hair has been grey for 25+ years and over the last 10 years he has noted worsening bifrontal hairline recession and occipital hair loss.

Current Medications

Aleve (Bayer Healthcare, naproxen sodium) p.r.n. for “arthritis”.

Physical Examination

T 97, BP 140/90, P 84, RR 20, weight 157 lbs, height 69 inches HEENT-carotids 2+ bilaterally, no bruits, JVD or adenopathy; sclera white, TM’s intact, pharynx, unremarkable, thyroid without nodules.

Chest: Symmetrical, lungs clear to percussion and auscultation, except bibasilar rales present.

Heart: S1, S2, regular rate and rhythm, no murmurs.

Extremities: No edema, no clubbing.

Laboratory and Imaging Studies

Laboratory tests: ANA positive 1:160, speckled pattern, Rheumatoid factor negative.

Baseline Chest x-ray: Bilateral, prominent interstitial markings consistent with pulmonary fibrosis.

High resolution CT scan of the chest: Diffuse ground glass changes extensively involving both lungs and all lobes, pathopneumonic for interstitial pulmonary fibrosis.

Baseline pulmonary function tests were also performed. (See Table 1).

Open lung biopsies were performed under general anesthesia and both adequate biopsy specimens showed substantial interstitial fibrotic change, an interstitial mononuclear cell infiltrate and a relative absence of eosinophils. No evidence of asbestosis was present on any of the numerous sections of tissue examined. Several pathologists were consulted and all agreed with the diagnosis of usual interstitial pneumonitis (UIP). Given the positive ANA, the high titer and pattern as well as the inflammatory infiltrate plus the family history of autoimmune disease, an autoimmune basis for this patient's interstitial

pulmonary fibrosis was thought to be possible. The relative absence of eosinophils made a hypersensitivity pneumonitis less likely [7].

Treatment

The patient was treated by mixing n-acetylcysteine 10% for inhalation, with a single vial of gamma interferon-1b (Actimmune®, InterMune, Inc.) containing 100 micrograms of gamma interferon-1b (2 million International Units per vial, 20 million International Units per milligram) in aqueous solution in 0.5 ml such that the total volume in the nebulizer reservoir was 20 ml. The solution was immediately delivered to the patient via a nebulized aerosol into his lungs. The patient was treated daily with one vial (100 mcg) of gamma interferon-1b in this way.

The dose of gamma interferon was increased by one vial per week, with the same diluent and dilution protocol, until side effects became a tolerable problem at 1000 micrograms (20 million International Units) per day.

The patient was then started on high dose intravenous immunoglobulin (HDIVIG) at a dose of 3.0 grams of immunoglobulin per kg of body weight every 3-8 weeks, for its anti-inflammatory activity [15,16], about ten weeks after starting the inhaled gamma interferon.

With the addition of HDIVIG, gamma interferon-1b side effects were greatly reduced. The dose of gamma interferon was increased by one vial per week until side effects again became a problem at 1,200 micrograms (24 million International Units) per day.

Post Treatment Test Results: Fourteen Months Post Treatment

Chest x-ray: Improvement of interstitial markings, normal lung parenchyma probably present.

High Resolution CT scan of the chest: Substantial improvement of ground glass changes and probably generation of new lung parenchyma.

Interestingly, the patient had grey scalp hair for over 25 years and about 5 months into treatment a portion of his hair roots turned blonde. Additionally, his hair has become thicker and he has lost almost all evidence of frontal and occipital thinning or recession. His thinking is sharp, he is working longer hours, and enjoying increased recreational activities. Some of these activities include long, brisk walks and his mood is very positive. The side effects of the treatment have been tolerable. Treatment was discontinued at 14 months with no recurrence of disease at two years. The patient's pulmonary function tests remained stable off treatment and the data strongly supports the hypothesis that new lung parenchyma had been generated.

Gamma interferon had previously failed in a clinical trial for pulmonary fibrosis [6]. In this study the drug was administered subcutaneously at a dose of 4 million International Units, three times per week.

In the patient described in this report a suprapharmacological dose of ultimately 24 million International Units was delivered via the off-label inhalation route. I hypothesize that to achieve potent fibrosis inhibition suprapharmacologic and local dosing of gamma interferon is necessary because the short serum half life of gamma interferon prohibits therapeutic levels at the site of disease or injury.

TABLES

Table 1. Baseline Pulmonary Function Tests obtained prior to beginning treatment.

Spirometry	Actual	Predicted	% Predicted
FVC (L)	2.14	3.66	59
FEV_1 (L)	1.82	2.83	64
FEV_3 (L)	2.14	3.35	64
$Dsb_{adj.}$ ml/min/mmHg	10.92	23.19	47
Va (L)	3.40	6.12	56

Table 2. Pulmonary Function Tests fourteen months after starting treatment.

Spirometry	Actual	Predicted	% Predicted
FVC (L)	2.74	3.66	75
FEV_1 (L)	3.86	2.83	136
TLC (L)	10.92	23.19	47
FRC (L)	3.40	6.12	56
RV (L)	2.71	2.63	103
VC (L)	3.70	3.66	101
IC (L)	1.82	2.37	76.7
ERV (L)	0.80	1.22	65.6
$Dsb_{adj.}$ ml/min/mmHg	21.19	23.19	91.4
Va (L)	4.06	6.12	66.3

Abbreviations: FVC, Forced Vital Capacity, Liters; FEV, Force Expiratory Volume in one second, Liters; FEV_3, Forced Expiratory Volume in three seconds, Liters; $Dsb_{adj.}$, Carbon Monoxide diffusing capacity, Ml/min/mmHg; Va, Alveolar Volume, Liters; TLC, Total lung Capacity, Liters; FRC, Functional Residual Capacity, Liters; RV, Residual Volume, Liters; VC, Vital Capacity, Liters; ERV, Expiratory Reserve Volume, Liters.

DISCUSSION

The currently available treatments for pulmonary fibrosis have not been shown to improve survival or to substantially improve lung function in well-designed multicenter, large clinical trials [7,9]. Moreover, these "standard" treatments which utilize corticosteroids and other immunosuppressants may increase morbidity and decrease quality of life and contribute to an increased risk of life threatening infection.

The subsets of patients who have extensive UIP disease are particularly difficult to treat [7,9]. Advanced age further worsens the prognosis in these patients. The patient's prognosis at the time of diagnosis was poor with an estimated life expectancy of 1-2 years and probable evolution to a need for home oxygen therapy within months. I was able to administer suprapharmacologic doses of IFN-gamma-1b directly into the lung with no substantial toxicity and few side effects to the patient. The doses are 10-25 fold greater than those used in other studies of this drug in humans, yet I have seen fewer side effects than would be expected, even when compared to the lower doses.

I have designed a sequential treatment regime escalating to very high dose inhaled IFN-gamma-1b plus HDIVIG which resulted in probable regeneration of functional alveoli. Regeneration of organs in adult humans, except for some potential for liver regeneration, has not been previously shown.

This patient had advanced UIP and had already lost much of his lung to the fibrotic (scarring) process. The loss of lung function was well documented by history, physical examination, multiple pulmonary function tests, multiple chest x-rays, an adequate two-specimen lung biopsy, and two high resolution CT scans. It is unheard of for such a patient to regain almost all his lung function (see Table 2) after it had been turned to "scar." I believe the only explanation for the dramatic results seen in the patient is that the therapy shut off the autoimmune and/or fibrotic response and induced regeneration of new lung parenchyma and probably new alveoli.

The patient required long term treatment with gamma interferon. I suspect that the scarred lung parenchyma needed to undergo collagen remodeling and in the gamma interferon induced milieu new lung parenchyma, including alveoli, developed. Shorter term gamma interferon administration might be sufficient if scarring (fibrosis) has not already developed.

Interestingly, the patient, despite being gray for 25 years has regained some blond hair pigment and has regrown almost all of his hair. This may represent *hair follicle stem cell* induction.

These very preliminary data suggest reversion, at least partially, to an embryonic developmental state at two sites, the lung and scalp.

Although the preliminary data in this report deals with lung regeneration, I believe that by delivering very high dose gamma interferon to other organs or tissues it may be possible to induce a regenerative response.

For example, in the case of an amputated digit the damaged end can be treated with a polymeric scaffold decorated with gamma interferon.

Treatment of the heart can use the same inhalational protocol described herein. Treatment of other organs can be accomplished via continuous infusion of gamma interferon via the arteries feeding the target organ.

COMPETING INTERESTS

The author declares that he has no competing interests.

ACKNOWLEDGEMENT

This paper is dedicated to the memory of Dr. Dan E. Pratt, a brilliant scientist and teacher.

REFERENCES

[1] Muneoka K, Han M, Gardiner DM. Regrowing human limbs. Sci Am. 2008 Apr;298(4):56-63.

[2] Gardiner DM. Ontogenetic decline of regenerative ability and the stimulation of human regeneration. Rejuvenation Res. 2005 Fall;8(3):141-53.

[3] Han M, Yang X, Taylor G, Burdsal CA, Anderson RA, Muneoka K. Limb regeneration in higher vertebrates: developing a roadmap. Anat Rec B New Anat. 2005 Nov;287(1):14-24.

[4] Brockes JP, Kumar A. Appendage regeneration in adult vertebrates and implications for regenerative medicine. Science. 2005 Dec 23;310(5756):1919-23.

[5] Sharpe RJ. Endogenous gamma interferon production may protect against hepatic cirrhosis and administration of exogenous gamma interferon may protect individuals prone to cirrhosis. Med Hypotheses. 1987 Apr;22(4):415-9.

[6] Kalra S, Utz JP, Ryu JH; Mayo Clinic Interstitial Lung Diseases Group. Interferon gamma-1b therapy for advanced idiopathic pulmonary fibrosis. Mayo Clin Proc. 2003 Sep;78(9):1082-7.

[7] American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med. 2000 Feb;161(2 Pt 1):646-64.

[8] Cooper JA Jr, White DA, Matthay RA. Drug-induced pulmonary disease. Part 1: Cytotoxic drugs. Am Rev Respir Dis. 1986 Feb;133(2):321-40.

[9] Araki T, Katsura H, Sawabe M, Kida K. A clinical study of idiopathic pulmonary fibrosis based on autopsy studies in elderly patients. Intern Med. 2003 Jun;42(6):483-9.

[10] Edwards SW, Say JE, Hughes V. Gamma interferon enhances the killing of Staphylococcus aureus by human neutrophils. J Gen Microbiol. 1988 Jan;134(1):37-42.

[11] Chacon RA, Corris PA, Dark JH, Gibson GJ. Comparison of the functional results of single lung transplantation for pulmonary fibrosis and chronic airway obstruction. Thorax. 1998 Jan;53(1):43-9.

[12] Sharpe RJ, Margolis RJ, Askari M, Amento EP, Granstein RD. Induction of dermal and subcutaneous inflammation by recombinant cachectin/tumor necrosis factor (TNF alpha) in the mouse. J Invest Dermatol. 1988 Oct;91(4):353-7.

[13] Farrell MM. The challenge of adult respiratory distress syndrome during interleukin-2 immunotherapy. Oncol Nurs Forum. 1992 Apr;19(3):475-80.

[14] Hack CE, Aarden LA, Thijs LG. Role of cytokines in sepsis. Adv Immunol. 1997;66:101-95.

[15] Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV. Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science. 2008 Apr 18;320(5874):373-6.

[16] Jolles S. A review of high-dose intravenous immunoglobulin (HDIVIg) in the treatment of the autoimmune blistering disorders. Clin Exp Dermatol. 2001 Mar;26(2):127-31.

[17] Hanson D, Winterbauer RH, Kirtland SH, Wu R. Changes in pulmonary function test results after 1 year of therapy as predictors of survival in patients with idiopathic pulmonary fibrosis. Chest. 1995 Aug;108(2):305-10.