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(54) Title: TREATMENT OF CARDIAC HYPERTROPHY

(57) Abstract

The invention concerns the treatment of cardiac hypertrophy by interferon-gamma (IFN-γ). Cardiac hypertrophy may result from a variety of diverse pathologic conditions, including myocardial infarction, hypertension, hypertrophic cardiomyopathy, and valvular regurgitation. The treatment extends to all stages of the progression of cardiac hypertrophy, with or without structural damage of the heart muscle, regardless of the underlying cardiac disorder.

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TREATMENT OF CARDIAC HYPERTROPHY

FIELD OF THE INVENTION

The present invention relates generally to the effects of IFN-γ on cardiac hypertrophy. More particularly, the invention concerns the use of IFN-γ for the prevention and treatment of cardiac hypertrophy and associated pathological conditions.

BACKGROUND OF THE INVENTION

Interferon-gamma (IFN-γ)

Interferons are relatively small, single-chain glycoproteins released by cells invaded by viruses or certain other substances. Interferons are presently grouped into three major classes, designated leukocyte interferon (interferon-alpha, α-interferon, IFN-α), fibroblast interferon (interferon-beta, β-interferon, IFN-β), and immune interferon (interferon-gamma, γ-interferon, IFN-γ). In response to viral infection, lymphocytes synthesize primarily α-interferon (along with a lesser amount of a distinct interferon species, commonly referred to as omega interferon), while infection of fibroblasts usually induces β-interferon. α- and β-interferons share about 20-30 percent amino acid sequence homology. The gene for human IFN-β lacks introns, and encodes a protein possessing 29% amino acid sequence identity with human IFN-α1, suggesting that IFN-α and IFN-β genes have evolved from a common ancestor (Taniguchi *et al.*, Nature 285, 547-549 [1980]). By contrast, IFN-γ is not induced by viral infection, rather, is synthesized by lymphocytes in response to mitogens, and is scarcely related to the other two types of interferons in amino acid sequence. Interferons-α and -β are known to induce MHC Class I antigens, while IFN-γ induces MHC Class II antigen expression, and also increases the efficiency with which target cells present viral peptide in association with MHC Class I molecules for recognition by cytotoxic T cells.

IFN-γ is a member of the interferon family, which exhibits the antiviral and anti-proliferative properties characteristic of interferons-α and -β (IFN-α and IFN-β) but, in contrast to those interferons, is PH 2 labile. IFN-γ was originally produced upon mitogenic induction of lymphocytes. The recombinant production of human IFN-γ was first reported by Gray, Goeddel and co-workers (Gray *et al.*, Nature 295, 503-508 [1982]), and is subject of U.S. Patent Nos. 4,762,791, 4,929,544, 4,727,138, 4,925,793, 4,855,238, 5,582,824, 5,096,705, 5,574,137, and 5,595,888. The recombinant human IFN-γ of Gray and Goeddel as produced in *E. coli*, consisted of 146 amino acids, the N-terminal position of the molecule commencing with the sequence CysTyrCys. It has later been found that the native human IFN-γ (i.e., that arising from mitogen induction of human peripheral blood lymphocytes and subsequent purification) is a polypeptide which lacks the CysTyrCys N-terminus assigned by Gray *et al.*, *supra*. More recently, the crystal structure of *E. coli* derived recombinant human IFN-γ (rhIFN-γ) was determined (Ealick *et al.*, Science 252, 698-702 [1991]), showing that the protein exists as a tightly intertwined non-covalent homodimer, in which the two identical polypeptide chains are oriented in an antiparallel manner.

IFN-γ is known to exhibit a broad range of biological activities, including antitumor, antimicrobial and immunoregulatory activities. A particular form of recombinant human IFN-γ (rhIFN-γ-1b, Actimmune®, Genentech, Inc. South San Francisco, California) is commercially available as an immunomodulatory drug for the treatment of chronic granulomatous disease characterized by severe, recurrent infections of the skin, lymph nodes, liver, lungs, and bones due to phagocyte disfunction (Baehner, R.L., Pediatric Pathol. 10, 143-153 [1990]). IFN-γ has also been proposed for the treatment of atopic dermatitis, a common inflammatory skin disease characterized by severe pruritus, a chronically relapsing course with frequent periods of exacerbation, a distinctive clinical

morphology and distribution of skin lesions (see PCT Publication No. WO 91/07984 published 13 June 1991), vascular stenosis, including the treatment of restenosis following angioplasty and/or vascular surgery (PCT Publication No. WO 90/03189 published 5 April 1990), various lung conditions, including respiratory distress syndromes (RDS), such as adult respiratory distress syndrome (ARDS) and a neonatal form, termed variously as idiopathic RDS or hyaline membrane disease (PCT Publication No. WO 89/01341, published 23 February 1989). In addition, IFN-γ has been proposed for use in the treatment of various allergies, e.g. asthma, and HIV-infection-related conditions, such as opportunistic infections, e.g. *Pneumocystis carinii* pneumonia, and trauma-associated sepsis. Impaired IFN-γ production has been observed in multiple-sclerosis (MP) patients, and it has been reported that the production of IFN-γ is greatly suppressed in suspensions of mitogen-stimulated mononuclear cells derived from AIDS patients. For a review see, for example, Chapter 16, "The Presence of Possible Pathogenic Role of Interferons in Disease". In: Interferons and other Regulatory Cytokines, Edward de Maeyer (1988, John Wilet and Sons Publishers).

Interferon-γ, along with other cytokines, has been implicated as an inducer of inducible nitric oxide (iNOS) which, in turn, has been described as an important mediator of the inflammatory mechanism underlying heart failure, of the cardiac response to sepsis or allograft rejection, as well as the progression of dilated cardiomyopathies of diverse etiologies. Ungureanu-Longrois *et al.*, Circ. Res. 77, 494-502 (1995); Pinsky *et al.*, J. Clin. Invest. 95, 677-685 (1995); Singh *et al.*, J. Biol. Chem. 270, 28471-8 (1995); Birks and Yacoub, Coronary Artery Disease 8, 389-402 (1997); Hattori *et al.*, J. Mol. Cell. Cardiol. 29, 1585-92 (1997). Indeed, IFN-γ has been reported to be the most potent single cytokine with regard to myocyte iNOS induction (Watkins *et al.*, J. Mol. & Cell. Cardiol. 27, 2015-29 [1995]).

Cardiac hypertrophy

Hypertrophy is generally defined as an increase in size of an organ or structure independent of natural growth that does not involve tumor formation. Hypertrophy of an organ or tissue is due either to an increase in the mass of the individual cells (true hypertrophy), or to an increase in the number of cells making up the tissue (hyperplasia), or both.

Cardiac hypertrophy is the enlargement of heart that is activated by both mechanical and hormonal stimuli and enables the heart to adapt to demands for increased cardiac output or to injury. Morgan and Baker, Circulation 83, 13-25 (1991). This response is frequently associated with a variety of distinct pathological conditions, such as hypertension, aortic stenosis, myocardial infarction, cardiomyopathy, valvular regurgitation, cardiac shunt, congestive heart failure, etc.

On a cellular level, the heart functions as a syncytium of myocytes and surrounding support cells, called non-myocytes. While non-myocytes are primarily fibroblast/mesenchymal cells, they also include endothelial and smooth muscle cells. Indeed, although myocytes make up most of the adult myocardial mass, they represent only about 30% of the total cell numbers present in heart.

The enlargement of embryonic heart is largely dependent on an increase in myocyte number, which continues until shortly after birth, when cardiac myocytes lose their proliferative capacity. Further growth occurs through hypertrophy of the individual cells. Hypertrophy of adult cardiac ventricular myocytes is a response to a variety of conditions which lead to chronic hemodynamic overload. Thus, in response to hormonal, physiological, hemodynamic, and pathological stimuli, adult ventricular muscle cells can adapt to increased workloads through

the activation of a hypertrophic process. This response is characterized by an increase in myocyte cell size and contractile protein content of individual cardiac muscle cells, without concomitant cell division and activation of embryonic genes, including the gene for atrial natriuretic peptide (ANP). Chien *et al.*, FASEB J. 5, 3037-3046 (1991); Chien *et al.*, Annu. Rev. Physiol. 55, 77-95 (1993). An increment in myocardial mass as a result of an increase in myocyte size that is associated with an accumulation of interstitial collagen within the extracellular matrix and around intramyocardial coronary arteries has been described in left ventricular hypertrophy secondary to pressure overload in humans (Caspari *et al.*, Cardiovasc. Res. 11, 554-8 [1977]; Schwarz *et al.*, Am. J. Cardiol. 42, 895-903 [1978]; Hess *et al.*, Circulation 63, 360-371 [1981]; Pearlman *et al.*, Lab. Invest. 46, 158-164 [1982]). Cardiac hypertrophy due to chronic hemodynamic overload is the common end result of most cardiac disorders and a consistent feature of cardiac failure.

It has also been suggested that paracrine factors produced by non-myocyte supporting cells may additionally be involved in the development of cardiac hypertrophy, and various non-myocyte derived hypertrophic factors, such as, leukocyte inhibitory factor (LIF) and endothelin, have been identified. Metcalf, Growth Factors 7, 169-173 (1992); Kurzrock *et al.*, Endocrine Reviews 12, 208-217 (1991); Inoue *et al.*, Proc. Natl. Acad. Sci. USA 86: 2863-2867 (1989); Yanagisawa and Masaki, Trends Pharm. Sci. 10, 374-378 (1989); U.S. Patent No. 5,573,762 (issued November 12, 1996). Further exemplary factors that have been identified as potential mediators of cardiac hypertrophy include cardiotrophin-1 (CT-1) (Pennica *et al.*, Proc. Nat. Acad. Sci. USA 92:1142-1146 [1995]), catecholamines, adrenocorticosteroids, angiotensin, and prostaglandins.

Adult myocyte hypertrophy is initially beneficial as a short term response to impaired cardiac function by permitting a decrease in the load on individual muscle fibers. With severe, long-standing overload, however, the hypertrophied cells begin to deteriorate and die. Katz, "Heart Failure", in: Katz A.M. ed., Physiology of the Heart (New York, Raven Press, 1992) pp. 638-668. Cardiac hypertrophy is a significant risk factor for both mortality and morbidity in the clinical course of heart failure. Katz, Trends Cardiovasc. Med. 5, 37-44 (1995).

For further details of the causes and pathology of cardiac hypertrophy see, e.g. Heart Disease, A Textbook of Cardiovascular Medicine, Braunwald, E. ed., W.B. Saunders Co., 1988. Chapter 14, Pathophysiology of Heart Failure.

Treatment of cardiac hypertrophy

At present, the treatment of cardiac hypertrophy varies depending on the underlying cardiac disease. Catecholamines, adrenocorticosteroids, angiotensin, prostaglandins, leukemia inhibitory factor (LIF), endothelin (including endothelin-1, -2, and -3 and big endothelin), cardiotrophin-1 (CT-1) and cardiac hypertrophy factor (CHF) are among the factors identified as potential mediators of hypertrophy. For example, β-adrenergic receptor blocking drugs (β-blockers, e.g., propranolol, timolol, tertalolol, carteolol, nadolol, betaxolol, penbutolol, acetobutolol, atenolol, metoprolol, carvedilol, etc.) and verapamil have been used extensively in the treatment of hypertrophic cardiomyopathy. The beneficial effects of β-blockers on symptoms (e.g. chest pain) and exercise tolerance are largely due to a decrease in the heart rate with a consequent prolongation of diastole and increased passive ventricular filling. Thompson *et al.*, Br. Heart J. 44, 488-98 (1980); Harrison *et al.*, Circulation 29, 84-98 (1964). Verapamil has been described to improve ventricular filling and probably reducing myocardial ischemia. Bonow *et al.*, Circulation 72, 853-64 (1985). Nifedipine and diltiazem have also been used occasionally in the treatment of hypertrophic cardiomyopathy. Lorell *et al.*, Circulation 65, 499-507 (1982); Betocchi *et al.*, Am. J.

Cardiol. 78, 451-7 (1996). However, because of its potent vasodilating properties, nifedipine may be harmful, especially in patients with outflow obstruction. Disopyramide has been used to relieve symptoms by virtue of its negative inotropic properties. Pollick, N. Engl. J. Med. 307, 997-9 (1982). In many patients, however, the initial benefits decrease with time. Wigle *et al.*, Circulation 92, 1680-92 (1995).

Antihypertensive drug therapy has been reported to have beneficial effects on cardiac hypertrophy associated with elevated blood pressure. Examples of drugs used in antihypertensive therapy, alone or in combination, are calcium antagonists, e.g. nitrendipine; β-adrenergic receptor blocking agents, e.g., those listed above; angiotensin converting enzyme (ACE) inhibitors, e.g., quinapril, captopril, enalapril, ramipril, benazepril, fosinopril, lisinopril; diuretics, e.g. chorothiazide, hydrochlorothiazide, hydroflumethazide, methylchlothiazide, benzthiazide, dichlorphenamide, acetazolamide, indapamide; calcium channel blockers, e.g. diltiazem, nifedipine, verapamil, nicardipine. For example, treatment of hypertension with diltiazem and captopril showed a decrease in left ventricular muscle mass, but the Doppler indices of diastolic function did not normalize. Szlachcic *et al.*, Am. J. Cardiol. 63, 198-201 (1989); Shahi *et al.*, Lancet 336, 458-61 (1990). These findings were interpreted to indicate that excessive amounts of interstitial collagen may remain after regression of left ventricular hypertrophy. Rossi *et al.*, Am. Heart J. 124, 700-709 (1992). Rossi *et al.*, *supra*, investigated the effect of captopril on the prevention and regression of myocardial cell hypertrophy and interstitial fibrosis in pressure overload cardiac hypertrophy, in experimental rats.

As there is no generally applicable therapy for the treatment of cardiac hypertrophy, the identification of factors that can prevent or reduce cardiac myocyte hypertrophy is of primary importance in the development of new therapeutic strategies to inhibit pathophysiological cardiac growth.

SUMMARY OF THE INVENTION

We have unexpectedly found that IFN-γ inhibits the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)- and phenylephrine-induced spreading of cardiac myocytes isolated from adult rats. We have further found that IFN-γ inhibits *in vivo* both cardiac hypertrophy induced by fluprostenol, an agonist analog of $PGF_{2\alpha}$, and hypertrophy induced by pressure overload in a rat model.

Accordingly, the present invention concerns the treatment of cardiac hypertrophy, regardless of the underlying cause, by administering a therapeutically effective dose of IFN-γ. If the objective is the treatment of human patients, IFN-γ preferably is recombinant human IFN-γ (rhIFN-γ), most preferably, rhIFN-γ-1b, which will be defined hereinbelow. The concept of treatment is used in the broadest sense, and specifically includes the prevention (prophylaxis), moderation, reduction, and curing of cardiac hypertrophy of any stage.

IFN-γ preferably is administered in the form of a liquid pharmaceutical formulation, which may be preserved to achieve extended storage stability. Preserved liquid pharmaceutical formulations might contain *multiple doses of IFN-γ, and might, therefore, be suitable for repeated use.*

IFN-γ might be administered in combination with one or more further therapeutic agent used for the treatment of cardiac hypertrophy, or a physiological condition instrumental in the development of cardiac hypertrophy, such as elevated blood pressure, aortic stenosis, or myocardial infarction.

The invention further concerns a method for making a pharmaceutical composition for the treatment of cardiac hypertrophy, which comprises IFN-γ as an active ingredient.

The invention also concerns a pharmaceutical product which comprises:

(a) a pharmaceutical composition comprising at least one therapeutically effective dosage of IFN-γ;

(b) a container containing said pharmaceutical composition; and

(c) a label affixed to said container, or a package insert included in said pharmaceutical product referring to the use of said IFN-γ in the treatment of cardiac hypertrophy.

BRIEF DESCRIPTION OF THE FIGURES

In the Figures and throughout the examples, "IFN" or "IFN-γ" refers to recombinant mouse IFN-γ (Genentech, Inc., South San Francisco, CA, or Genzyme, Cambridge, MA).

Figure 1 Inhibition of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced spreading response by IFN-γ. Myocytes were pre-incubated with saline vehicle or IFN-γ (500 U/ml) on day of isolation. A second addition of vehicle or IFN-γ was performed 24h after isolation, along with the addition of either vehicle or $PGF_{2\alpha}$ (10^{-7} M). After an additional 72 hr incubation, cells were fixed in glutaraldehyde, stained with eosin Y and viewed by fluorescence microscopy. A, B, C Cardiac myocytes after 4 days in culture: control, $PGF_{2\alpha}$, and $PGF_{2\alpha}$+IFN-γ, respectively. Histographs showing maximum breadth of rod shaped cardiac myocytes versus percent frequency of breadth occurrence. The maximum breadth of rod-shaped cells was determined by fluorescence microscopy and imaging software. At least 200 rod shaped cells from a single experiment were examined per group. IFN-γ alone had no observable effect on the morphology of the cells. $P<0.001$ for all group comparisons.

Figure 2 Dose responsive inhibition of $PGF_{2\alpha}$-induced response by IFN-γ (500-25 U/ml). Myocytes were pre-incubated with saline vehicle or IFN-γ on day of isolation. A second amount of IFN-γ was added 24 hr after isolation, along with the addition of either vehicle or $PGF_{2\alpha}$ (10^{-7} M). After an additional 72 hr incubation, cells were fixed in glutaraldehyde, stained with eosin Y and viewed under fluorescence. Quantitation of myocyte morphology: A control, B $PGF_{2\alpha}$, C $PGF_{2\alpha}$+IFN-γ(25 U/ml), D $PGF_{2\alpha}$ + IFN-γ (100 U/ml), E $PGF_{2\alpha}$+IFN-γ(500 U/ml). Histographs showing maximum breadth of rod shaped cardiac myocytes versus percent frequency of breadth occurrence. The maximum breadth of rod-shaped cells was determined by fluorescence microscopy and imaging software. At least 200 rod shaped cells from a single experiment were examined per group. IFN-γ alone had no observable effect on the morphology of the cells. $P<0.001$ for all group comparisons.

Figure 3 Inhibition of phenylephedrine (PE)-induced spreading response by IFN-γ. Myocytes were pre-incubated with saline vehicle or IFN-γ (500 U/ml) on day of isolation. A second addition of vehicle or IFN-γ was performed 24h after isolation, along with the addition of either vehicle or PE (10^{-5} M). After an additional 72 hr incubation, cells were fixed in glutaraldehyde, stained with eosin Y and viewed by fluorescence microscopy. A, B, C Cardiac myocytes after 4 days in culture: control, PE, and PE+IFN-γ, respectively. Histographs showing maximum breadth of rod shaped cardiac myocytes versus percent frequency of breadth occurrence. The maximum breadth of rod-shaped cells was determined by fluorescence microscopy and imaging software. At least 200 rod shaped cells from a single experiment were examined per group. IFN-γ alone had no observable effect on the morphology of the cells. $P<0.001$ for all group comparisons.

Figure 4 Effects of IFN-γ on cardiac hypertrophy induced by fluprostenol in rats. Data are presented as mean ± SEM. The number in parentheses is the number of animals in each group. * $P<0.05$, *$P<0.01$, compared to the vehicle group. #$P<0.05$, ##$P<0.01$, compared to the Flup group. Flup: fluprostenol; IFN=IFN-γ; HW: heart weight; BW: body weight; VW: ventricular weight; LVW: left ventricular weight.

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Figure 5 Effects of Flup and/or IFN on MAP and HR. Data are presented as mean ± SEM. The number in parenthesis is the number of animals in each group. * P<0.05, compared to the vehicle group. # P<0.05, compared to the Flup group. + P<0.05, compared to the Flup+IFN group. Flup: fluoprostenol; IFN: IFN-γ; MAP: mean arterial pressure; HR: heart rate.

Figure 6 Bargraphs showing the effect of fluprostenol (FLUP) and IFN-γ on: A Skeletal actin (SKA); B Sarcoplasmic reticulum calcium ATPase (SRCA); C Collagen I (COL I); D Atrial natriuretic factor (ANF) expression. Expression levels are normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) message. VEH is vehicle. There were 7 animals per group and the data are presented as the mean ± SEM. P<0.05 vs VEH group.

Figure 7 Effects of IFN-γ on heart weight, ventricular weight, and left ventricular weight in rats with pressure overload. Data are presented as mean ± SEM. The number in parenthesis is the number of animals in each group. ** P<0.01, compared to the sham group. ## P<0.01, compared to the Banded+vehicle group. Sham: sham-operated rats; Banded: aortic-banded rats; IFN: IFN-γ; HW: heart weight; VW: ventricular weight; LVW: left ventricular weight.

Figure 8 Effects of IFN-γ on the ratio of heart weight, ventricular weight, and left ventricular weight to body weight in rats with pressure overload. Data are presented as mean ± SEM. The number in parenthesis is the number of animals in each group. **P<0.01, compared to the sham group. ##P<0.01, compared to the Banded+vehicle group. Sham: sham-operated rats; Banded: aortic-banded rats; IFN: IFN-γ; HW: heart weight; BW: body weight; VW: ventricular weight; LVW: left ventricular weight.

Figure 9 Effects of IFN-γ on systolic arterial pressure, mean arterial pressure, and diastolic arterial pressure in rats with pressure overload. The number in parenthesis is the number of animals in each group. **P<0.01, compared to the sham group. Sham: sham-operated rats; Banded: aortic-banded rats; IFN: IFN-γ, SAP: systolic arterial pressure; MAP: mean arterial pressure; DAP: diastolic arterial pressure.

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

"Gamma interferon", "interferon-gamma", or "IFN-γ" refers variously to all forms of (human and non-human animal) gamma interferon that are shown to be biologically active in any assay of cardiac hypertrophy, e.g. the hypertrophy assays disclosed herein, and is meant to include, but is not limited to, mature, pro, met and/or des(1-3) (also referred to as desCysTyrCys IFN-γ) form, whether obtained from natural source, chemically synthesized or produced by techniques of recombinant DNA technology. A complete description of the preparation of recombinant human IFN-γ (rhuIFN-γ) including its cDNA and amino acid sequences are disclosed, for example, in United States Patent Nos. 4,727,138; 4,762,791; 4,925,793; 4,929,554; 5,582,824; 5,096,705; 4,855,238; 5,574,137; and 5,595,888. CysTyrCys-lacking recombinant human IFN-γ, including variously truncated derivatives are, for example, disclosed in European Patent Publication No. 146,354. Non-human animal interferons, including IFN-γ, are, for example, disclosed in European Publication No. 88,622. The term includes variously glycosylated forms and other variants (e.g. amino acid sequence variants) and derivatives of such native (wild-type) interferons, whether known in the art or will become available in the future. Examples of such variants are alleles, and the products of site-directed mutagenesis in which residues are deleted, inserted and/or substituted (see, e.g. European Publication No. 146,354 referred to above). IFN-γ is known to have a narrow host range, therefore, IFN-γ

homologous to the animal to be treated should be used. In human therapy, the desCysTyrCys variant of the sequence shown in U. S. Patent No. 4,717,138 and its counterpart, EP 77,670, is preferably employed, and optionally the C-terminal variant in which the last four amino acid residues are deleted in post-translational processing. For human therapeutic use, the IFN-γ of the present invention preferably is recombinant human IFN-γ (rhIFN-γ), with or without the amino acids CysTyrCys at the N-terminus. More preferably, IFN-γ is a recombinant human IFN-γ species (recombinant human interferon gamma-1b, rhIFN-γ-1b, containing 140 amino acids), which is the active ingredient of the commercial formulation, Actimmune® (Genentech, Inc., South San Francisco, California). As IFN-γ is known to be highly species specific, in animal experiments, or for veterinary use, IFN-γ of the animal species to be treated is preferably employed. Thus, in the *in vivo* experiments using a rat animal model, murine (mouse) recombinant IFN-γ (Genentech, Inc.) has been employed. Rat and mice and sufficiently closely related to permit the use of mouse IFN-γ in a rat model.

In a pharmacological sense, in the context of the present invention, a "therapeutically effective amount" of IFN-γ refers to an amount effective in the treatment of hypertrophy, specifically cardiac hypertrophy.

"Hypertrophy", as used herein, is defined as an increase in mass of an organ or structure independent of natural growth that does not involve tumor formation. Hypertrophy of an organ or tissue is due either to an increase in the mass of the individual cells (true hypertrophy), or to an increase in the number of cells making up the tissue (hyperplasia), or both. Certain organs, such as the heart, lose the ability to divide shortly after birth. Accordingly, "cardiac hypertrophy" is defined as an increase in mass of the heart, which, in adults, is characterized by an increase in myocyte cell size and contractile protein content without concomitant cell division. The character of the stress responsible for inciting the hypertrophy, (e.g., increased preload, increased afterload, loss of myocytes, as in myocardial infarction, or primary depression of contractility), appears to play a critical role in determining the nature of the response. The early stage of cardiac hypertrophy is usually characterized morphologically by increases in the size of mycrofibrils and mitochondria, as well as enlargement of mitochondria and nuclei. At this stage, while muscle cells are larger than normal, cellular organization is largely preserved. At a more advanced stage of cardiac hypertrophy, there are preferential increases in the size or number of specific organelles, such a mitochondria, and new contractile elements are added in localized areas of the cells, in an irregular manner. Cells subjected to long-standing hypertrophy show more obvious disruptions in cellular organization, including markedly enlarged nuclei with highly lobulated membranes, which displace adjacent myofibrils and cause breakdown of normal Z-band registration. The phrase "cardiac hypertrophy" is used to include all stages of the progression of this condition, characterized by various degrees of structural damage of the heart muscle, regardless of the underlying cardiac disorder.

"Heart failure" refers to an abnormality of cardiac function where the heart does not pump blood at the rate needed for the requirements of metabolizing tissues. The heart failure can be caused by a number of factors, including ischemic, congenital, rheumatic, or idiopathic forms.

"Congestive heart failure" is a progressive pathologic state where the heart is increasingly unable to supply adequate cardiac output (the volume of blood pumped by the heart over time) to deliver the oxygenated blood to peripheral tissues. As congestive heart failure progresses, structural and hemodynamic damages occur. While these damages have a variety of manifestations, one characteristic symptom is ventricular hypertrophy. Congestive heart failure is a common end result of a number of various cardiac disorders.

"Myocardial infarction" generally results from atherosclerosis of the coronary arteries, often with superimposed coronary thrombosis. It may be divided into two major types: transmural infarcts, in which myocardial necrosis involves the full thickness of the ventricular wall, and subendocardial (nontransmural) infarcts, in which the necrosis involves the subendocardium, the intramural myocardium, or both, without extending all the way through the ventricular wall to the epicardium. Myocardial infarction is known to cause both a change in hemodynamic effects and an alteration in structure in the damaged and healthy zones of the heart. Thus, for example, myocardial infarction reduces the maximum cardiac output and the stroke volume of the heart. Also associated with myocardial infarction is a stimulation of the DNA synthesis occurring in the interstice as well as an increase in the formation of collagen in the areas of the heart not affected.

As a result of the increased stress or strain placed on the heart in prolonged hypertension due, for example, to the increased total peripheral resistance, cardiac hypertrophy has long been associated with "hypertension". A characteristic of the ventricle that becomes hypertrophic as a result of chronic pressure overload is an impaired diastolic performance. Fouad *et al.*, J. Am. Coll. Cardiol. 4, 1500-6 (1984); Smith *et al.*, J. Am. Coll. Cardiol. 5, 869-74 (1985). A prolonged left ventricular relaxation has been detected in early essential hypertension, in spite of normal or supranormal systolic function. Hartford *et al.*, Hypertension 6, 329-338 (1984). However, there is no close parallelism between blood pressure levels and cardiac hypertrophy. Although improvement in left ventricular function in response to antihypertensive therapy has been reported in humans, patients variously treated with a diuretic (hydrochlorothiazide), a β-blocker (propranolol), or a calcium channel blocker (diltiazem), have shown reversal of left ventricular mass, without improvement in diastolic function. Inouye *et al.*, Am. J. Cardiol. 53, 1583-7 (1984).

Another complex cardiac disease associated with cardiac hypertrophy is "hypertrophic cardiomyopathy". This condition is characterized by a great diversity of morphologic, functional, and clinical features (Maron *et al.*, N. Engl. J. Med. 316, 780-9 [1987]; Spirito *et al.*, N. Engl. J. Med. 320, 749-55 [1989]; Louie and Edwards, Prog. Cardiovasc. Dis. 36, 275-308 [1994]; Wigle *et al.*, Circulation 92, 1680-92 [1995]), the heterogeneity of which is accentuated by the fact that it afflicts patients of all ages (Spirito *et al.*, N. Engl. J. Med. 336, 775-785 [1997]). The causative factors of hypertrophic cardiomyopathy are also diverse and little understood. Recent data suggest that β-myosin heavy chain mutations may account for approximately 30 to 40 percent of cases of familial hypertrophic cardiomyopathy (Watkins *et al.*, N. Engl. J. Med. 326, 1108-14 [1992] Schwartz *et al.* Circulation 91, 532-40 [1995]; Marian and Roberts, Circulation 92, 1336-47 [1995]; Thierfelder *et al.*, Cell 77, 701-12 [1994]; Watkins *et al.*, Nat. Gen. 11, 434-7 [1995]).

Supravalvular "aortic stenosis" is an inherited vascular disorder, that is characterized by narrowing of the ascending aorta, but other arteries, including the pulmonary arteries, may also be affected. Untreated aortic stenosis may lead to increased intracardiac pressure resulting in myocardial hypertrophy and eventually heart failure and death. The pathogenesis of this disorder is not fully understood, but hypertrophy and possibly hyperplasia of medial smooth muscle are prominent features of this disorder. It has been reported that molecular variants of the elastin gene are involved in the development and pathogenesis of aortic stenosis. (U.S. Patent No. 5,650,282 issued July 22, 1997.)

"Valvular regurgitation" occurs as a result of heart diseases resulting in disorders of the cardiac valves. Various diseases, like rheumatic fever, can cause the shrinking or pulling apart of the valve orifice, while other

diseases may result in endocarditis, an inflammation of the endocardium or lining membrane of the atrioventricular orifices and operation of the heart. Defects such as the narrowing of the valve stenosis or the defective closing of the valve result in an accumulation of blood in the heart cavity or regurgitation of blood past the valve. If uncorrected, prolonged valvular stenosis or insufficiency may result in cardiac hypertrophy and associated damage to the heart muscle, which may eventually necessitate valve replacement.

The treatment of all these, and other cardiac disorders accompanied by cardiac hypertrophy is subject of the present invention.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) hypertrophy. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. The hypertrophy may result from any cause, including idiopathic, cardiotrophic, or myotrophic causes, or ischemia or ischemic insults, such as myocardial infarction.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial antihypertrophic effect for an extended period of time.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cows, horses, sheep, pigs, etc. Preferably, the mammal is human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

B. Modes of Carrying out the Invention

1. **Cardiac hypertrophy assays**

In vitro assays

a. Induction of spreading of adult rat cardiac myocytes

In this assay, ventricular myocytes are isolated from a single (male Sprague-Dawley) rat, essentially following a modification of the procedure described in detail by Piper *et al.*, "Adult ventricular rat heart muscle cells." In: Cell Culture Techniques in Heart and Vessel Research, H.M. Piper, ed., Berlin: Spinger-Verlag, 1990, pp. 36-60. This procedure permits the isolation of adult ventricular myocytes and the long-term culture of these cells in the rod-shaped phenotype. Phenylephrine and Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) have been shown to induce a spreading response in these adult cells. Piper *et al., supra*; Lai *et al.*, Am J. Physiol. 1996; 271 (Heart Circ. Physiol. 40):H2197-H2208. The inhibition of myocyte spreading induced by $PGF_{2\alpha}$, or $PGF_{2\alpha}$ analogs, (e.g. fluprostenol) and phenylephrine by various potential inhibitors of cardiac hypertrophy is then tested. A detailed protocol is described in the examples that follow.

In vivo assays

a. Inhibition of cardiac hypertrophy induced by fluprostenol *in vivo*

This pharmacological model tests the ability of IFN-γ to inhibit cardiac hypertrophy induced in rats (e.g. male Wistar or Sprague-Dawley) by subcutaneous injection of fluprostenol (an agonist analog of $PGF_{2\alpha}$). It is known that rats with pathologic cardiac hypertrophy induced by myocardial infarction have chronically elevated levels of extractable $PGF_{2\alpha}$ in their myocardium. Lai *et al.*, Am. J. Physiol. (Heart Circ. Physiol.) 271:H2197-H2208 (1996). Accordingly, factors that can inhibit the effects of fluprostenol on myocardial growth *in vivo* are

potentially useful for treating cardiac hypertrophy. The effects of IFN-γ on cardiac hypertrophy are determined by measuring the weight of heart, ventricles, and left ventricle (normalized by bodyweight) relative to fluprostenol-treated rats not receiving IFN-γ. A detailed description of this assay is provided in the examples.

b. Pressure-overload cardiac hypertrophy assay.

For *in vivo* testing it is common to induce pressure-overload cardiac hypertrophy by constriction of the abdominal aorta of test animals. In a typical protocol rats (e.g. male Wistar or Sprague-Dawley) are treated under anesthesia, and the abdominal aorta of each rat is narrowed down just below the diaphragm. Beznak M., Can. J. Biochem. Physiol. 33, 985-94 (1955). The aorta is exposed through a surgical incision, and a blunted needle is placed next to the vessel. The aorta is constricted with a ligature of wool thread around the needle, which is immediately removed and which reduces the lumen of the aorta to the diameter of the needle. This approach is described, for example, in Rossi *et al.*, Am. Heart J. 124, 700-709 (1992) and O'Rourke and Reibel, P.S.E.M.B. 200, 95-100 (1992). A detailed description of the protocol used by the present inventors is disclosed in the examples hereinbelow.

b. Effect on cardiac hypertrophy following experimentally induced myocardial infarction (MI).

Acute MI is induced in rats by left coronary artery ligation and confirmed by electrocardiographic examination. A sham-operated group of animals is also prepared as control animals. Earlier data have shown that cardiac hypertrophy is present in the group of animals with MI, as evidenced by an 18% increase in heart weight-to-body weight ratio. Lai *et al.*, *supra*. Treatment of these animals with candidate blockers of cardiac hypertrophy, e.g. IFN-γ provides valuable information about the therapeutic potential of the candidates tested.

2. **Uses, therapeutic compositions and administration of IFN-γ**

In accordance with the present invention, IFN-γ can be used for the treatment of cardiac hypertrophy, i.e. the enlargement of heart, regardless of the etiology and pathogenesis. When an excessive pressure or volume load is imposed on the heart (ventricle), cardiac (myocardial) hypertrophy develops, providing a fundamental compensatory mechanism that permits the ventricle to sustain its burden. Krayenbuehl *et al.*, Eur. Heart J. 4 (Suppl. A), 29 (1983). The character of the stress (increased preload, increased afterload, loss of myocytes, as in myocardial infarction, or primary depression of contractility) responsible of the development of hypertrophy plays a critical role in determining the nature of the hypertrophic response. Scheuer and Buttrick, Circulation 75(Suppl. 1), 63 (1987). The present invention concerns the treatment of cardiac hypertrophy associated with any underlying pathological condition, including, without limitation, post myocardial infarction, hypertension, aortic stenosis, cardiomyopathy, valvular regurgitation, cardiac shunt, and congestive heart failure. The main characteristics of these conditions have been discussed hereinabove.

Particularly important is the use of IFN-γ for the prevention of cardiac failure following myocardial infarction. About 750,000 patients suffer from acute myocardial infarction (AMI) annually, and approximately one-fourth of all death in the United States are due to AMI. In recent years, thrombolytic agents, e.g. streptokinase, urokinase, and in particular tissue plasminogen activator (t-PA) have significantly increased the survival of patients who suffered myocardial infarction. When administered as a continuous intravenous infusion over 1.5 to 4 hours, t-PA produces coronary patency at 90 minutes in 69% to 90% of the treated patients. Topol *et al.*, Am. J. Cardiol. 61, 723-8 (1988); Neuhaus *et al.*, J. Am. Coll. Cardiol. 12, 581-7 (1988); Neuhaus *et al.*, J. Am. Col. Cardiol. 14, 1566-9 (1989). The highest patency rates have been reported with high dose or accelerated dosing regimens.

Topol, J. Am. Coll. Cardiol. 15, 922-4 (1990). t-PA may also be administered as a single bolus, although due to its relatively short half-life, it is better suited for infusion therapy. Tebbe *et al.*, Am. J. Cardiol. 64, 448-53 (1989). A t-PA variant, specifically designed to have longer half-life and very high fibrin specificity, TNK t-PA (a T103N, N117Q, KHRR(296-299)AAAA t-PA variant, Keyt *et al.*, Proc. Natl. Acad. Sci. USA 91, 3670-3674 (1994)) is particularly suitable for bolus administration. However, despite all these advances, the long-term prognosis of patient survival depends greatly on the post-infarction monitoring and treatment of the patients, which should include monitoring and treatment of cardiac hypertrophy.

Another important therapeutic indication is the treatment of cardiac hypertrophy associated with hypertension. As noted before, sustained hypertension is known to result in cardiac hypertrophy. Although certain hypotensive agents have been shown to reduce left ventricular mass, treatment does not always resulted in the improvement of diastolic function. Accordingly, IFN-γ can be administered in combination with β-adrenergic receptor blocking agents, e.g., propranolol, timolol, tertalolol, carteolol, nadolol, betaxolol, penbutolol, acetobutolol, atenolol, metoprolol, carvedilol; angiotensin converting enzyme (ACE) inhibitors, e.g., quinapril, captopril, enalapril, ramipril, benazepril, fosinopril, lisinopril; diuretics, e.g. chorothiazide, hydrochlorothiazide, hydroflumethazide, methylchlothiazide, benzthiazide, dichlorphenamide, acetazolamide, indapamide; and/or calcium channel blockers, e.g., diltiazem, nifedipine, verapamil, nicardipine. Pharmaceutical compositions comprising the therapeutic agents identified here by their generic names are commercially available, and are to be administered following the manufacturers' instructions for dosage, administration, adverse effects, contraindications,etc. (See, e.g. Physicians' Desk Reference, Medical Economics Data Production Co., Montvale, N.J., 51th Edition, 1997.)

IFN-γ may also be administered prophylactically to patients with cardiac hypertrophy, to prevent the progression of the condition, and avoid sudden death, including death of asymptomatic patients. Such preventative therapy is particularly warranted in the case of patients diagnosed with massive left ventricular cardiac hypertrophy (a maximal wall thickness of 35 mm or more in adults, or a comparable value in children), or in instances when the hemodynamic burden on the heart is particularly strong.

IFN-γ may also be useful in the management of atrial fibrillation, which develops in a substantial portion of patients diagnosed with hypertrophic cardiomyopathy.

IFN-γ is administered in the form of a pharmaceutical composition comprising IFN-γ as an active ingredient, in conjunction with a pharmaceutically acceptable carrier. Therapeutic formulations of IFN-γ for treating cardiac hypertrophy are prepared for storage by mixing IFN-γ having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, *supra)*, in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients, or stabilizers are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; 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, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics, or polyethylene glycol (PEG).

IFN-γ to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. IFN-γ ordinarily will be stored in lyophilized form or in solution.

IFN-γ may be used in lyophilized form, in combination with other ingredients for reconstitution with an appropriate diluent at the time for use. Because IFN-γ is known to be acid labile, it has traditionally been handled at neutral or slightly alkaline pH. See, for example, U.S. Patent No. 4,499,014 which discloses reactivation of a lyophilized acidic IFN-γ solution to a pH of 6 to 9. Neutral or slightly alkaline solutions of higher concentrations of IFN-γ are generally unsuitable as injectable formulations because of the immediate formation of a visible precipitate. Such precipitate may cause thrombosis on administration or decrease potency. European Patent Publication No. 0196,203 discloses reconstitution of lyophilized IFN-γ to a pH of 4 to 6.0.

Stable liquid pharmaceutical compositions comprising an effective amount of non-lyophilized IFN-γ along with a buffer capable of maintaining the pH at 4.0–6.0, a stabilizing agent, and a non-ionic detergent are disclosed in U.S. Patent No. 5,151,265 issued 29 September 1992. The stabilizing agent typically is a polyhydric sugar alcohol, such as mannitol, and the non-ionic detergent may be a surfactant, e.g. polysorbate 80 or polysorbate 20. The non-ionic detergent preferably is present in a range of about 0.07 to 0.2 mg/ml, and most preferably in a concentration of about 0.1 mg/ml. Suitable buffers are conventional buffers of organic acids and salt thereof, such as nitrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), succinate buffers (e.g., succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, etc.), tartarate buffers (e.g., tartaric acid-sodium tartarate mixture, tartaric acid-potassium tartarate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (e.g. fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (e.g. gluconic acid-sodium gluconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium gluconate mixture, etc.), oxalate buffers (e.g., oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, etc.), lactate buffers (e.g. lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.), and acetate buffers (e.g., acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.)

A known commercial liquid formulation of IFN-γ (Actimmune® rhuIFN-γ-1b, Genentech, Inc.) is a sterile, clear, colorless unpreserved solution filled in a single-dose vial for subcutaneous injection. Each 0.5 ml vial of Actimmune contains 100 µg (3 million U, specific activity: 30 million U/mg) of IFN-γ-1b formulated in 20 mg mannitol, 0.36 mg sodium succinate, 0.05 mg polysorbate 20 and Sterile Water for Injection.

Preserved pharmaceutical compositions to be used in accordance with the present invention, which are suitable for repeated use, preferably contain:

a) IFN-γ not subjected to prior lyophilization;

b) an acetate buffer capable of maintaining the pH between about 4 and about 6 (the pH range of maximum stability of the protein in solution);

c) a non-ionic detergent primarily to stabilize the protein against agitation-induced aggregation;

d) an isotonifier;

e) a preservative selected from the group of phenol, benzyl alcohol and a benzethonium halide, e.g. chloride; and

f) water.

The non-ionic detergents (surfactants) may, for example, be polysorbates (e.g. polysorbate [Tween] 20, 80, etc.) or poloxamers (e.g. poloxamer 188). The use of non-ionic surfactants permits the formulation to be exposed to shear surface stresses without causing denaturation of the protein. Further, such surfactant containing formulations may be employed in aerosol devices such as those used in a pulmonary dosing, and needleless jet injector guns (see, e.g. EP 257,956).

The isotonifier is present to ensure isotonicity of the liquid compositions of the present invention, and includes polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. These sugar alcohols can be used alone or in combination. Alternatively, sodium chloride or other appropriate inorganic salts may be used to render the solutions isotonic.

The acetate buffer may, for example, be an acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc. The pH of the liquid formulation of this invention is buffered in the range of about 4.0 to 6.0, preferably 4.5 to 5.5, and most preferably at about pH 5.

The preservatives phenol, benzyl alcohol and benzethonium halides, e.g. chloride, are known antimicrobial agents.

In a preferred embodiment, IFN-γ is administered in the form of a liquid pharmaceutical composition which comprises the following components:

IFN-γ	0.1-2.0 mg/ml
sodium acetate (pH 5.0)	5-100 mM
Tween 20	0.1 to 0.01 % by weight
phenol	0.05 to 0.4 % by weight
mannitol	5 % by weight
water for injection, USP	up to 100 %,

wherein the percentage amounts are based on the weight of the composition. Phenol can be replaced by 0.5-1.0 % by weight of benzyl alcohol, and mannitol can be replaced by 0.9 % by weight sodium chloride.

Most preferably, the compositions comprise

IFN-γ	0.1 to 1.0 mg/ml
sodium acetate (pH 5.0)	10 mM
Tween 20	0.01 % by weight
phenol	0.2 %
mannitol	5 %

Phenol can be replaced by 0.75 by weight benzyl alcohol and mannitol by 0.9 % by weight sodium chloride.

The preserved liquid formulations preferably contain multiple doses of a therapeutically effective amount of IFN-γ. In view of the narrow host range of this polypeptide, for the treatment of human patients liquid formulations comprising human IFN-γ, more preferably native sequence human IFN-γ, are preferred. As a biological response modifier, IFN-γ exerts a wide variety of activities on a wide range of cell types, in a variety of

human and non-human mammalian species. The therapeutically effective dose will, of course, vary depending on such factors as the pathological condition to be treated (including prevention), the patient's age, weight, general medical condition, medical history, etc., and its determination is well within the skill of a practicing physician. The effective dose generally is within the range of from about 0.001 to about 1.0 mg/kg, more preferably about 0.01-1 mg/kg, most preferably about 0.01-0.1 mg/kg. In such formulations huIFN-γ will preferably exhibit a specific activity of on the order of about 2 x 10^7 U/mg of protein or greater when tested on A549 cells against encephalomyocarditis virus. It should be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein. Moreover, for human administration, the liquid formulations should meet sterility, pyrogenicity, general safety, and purity as required by FDA Office and Biologics standards.

The route of IFN-γ administration is in accord with known methods, *e.g.*, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional routes, or by sustained-release systems as noted below. Therapeutic IFN-γ compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle. The formulations are preferably administered as repeated intravenous (i.v.), subcutaneous (s.c.) or intramuscular (i.m.) injections, or as aerosol formulations suitable for intranasal or intrapulmonary delivery (for intrapulmonary delivery see, e.g. EP 257,956).

The stable aqueous compositions of IFN-γ are preferably contained in vials, containing up to about 30 therapeutically effective doses of IFN-γ. The bioactivity of IFN-γ preferably remains within about 20 % from the bioactivity exhibited at the time of first administration for at least about 14 days, more preferably for at least about 200 days following first administration.

IFN-γ can also be administered in the form of sustained-released preparations. Suitable examples of sustained-release preparations include 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).

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated proteins remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for protein stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release IFN-γ compositions also include liposomally entrapped IFN-γ. Liposomes containing IFN-γ are prepared by methods known *per se*: DE 3,218,121; Epstein *et al.*, Proc. Natl. Acad. Sci. USA, 82: 3688-3692 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. USA, 77: 4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the optimal therapy.

An effective amount of IFN-γ to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. The recommended dosage for the administration of IFN-γ (Actimmune®, Genentech, Inc.) to treat patients with chronic granulomatous disease is 50 mcg/m^2 (1.5 million U/m^2) for patients whose body surface area is greater than 0.5 m^2, and 1.5 mcg/kg/dose for patients whose body surface area is equal to or less than 0.5 m^2, administered as a subcutaneous injection, three times a week. This is valuable guidance for a physician to determine the optimal effective dose for the treatment of cardiac hypertrophy. The clinician will administer IFN-γ until a dosage is reached that achieves the desired effect for treatment of the heart dysfunction. For example, if the objective is the treatment of congestive heart failure, the amount would be one which inhibits the progressive cardiac hypertrophy associated with this condition. The progress of this therapy is easily monitored by echo cardiography. Similarly, in patients with hypertrophic cardiomyopathy, IFN-γ can be administered on an empirical basis, relying on the patient's subjective perception of benefit.

IFN-γ may be administered in combination with other therapeutic agents used for the treatment (including prevention) of cardiac hypertrophy. For example, IFN-γ therapy can be combined with the administration of inhibitors of known cardiac myocyte hypertrophy factors, e.g. inhibitors of α-adrenergic agonists, e.g. phenylephrine; endothelin-1; CT-1; LIF; angiotensin converting enzyme, and angiotensin II. Inhibitors of cardiac hypertrophy factor (CHF, cardiotrophin or cardiotrophin-1, see, e.g. US 5,679,545) are particularly preferred for combination therapy.

Preferred candidates for combination therapy in the treatment of hypertrophic cardiomyopathy are β-adrenergic-blocking drugs (e.g., propranolol, timolol, tertalolol, carteolol, nadolol, betaxolol, penbutolol, acetobutolol, atenolol, metoprolol, carvedilol), verapamil, difedipine, diltiazem. Treatment of hypertrophy associated with high blood pressure may require the use of antihypertensive drug therapy, using calcium channel blockers, e.g. diltiazem, nifedipine, verapamil, nicardipine; β-adrenergic blocking agents; diuretics, e.g., chorothiazide, hydrochlorothiazide, hydroflumethazide, methylchlothiazide, benzthiazide, dichlorphenamide, acetazolamide, indapamide; and/or ACE-inhibitors, e.g., quinapril, captopril, enalapril, ramipril, benazepril, fosinopril, lisinopril.

The effective amount of the therapeutic agents administered in combination with IFN-γ will be at the physician's or veterinarian's discretion. Dosage administration and adjustment is done to achieve optimal management of the conditions to be treated, and ideally takes into account use of diuretics or digitalis, and conditions such as hyper- or hypotension, renal impairment, etc. The dose will additionally depend on such factors

as the type of the therapeutic agent to be used and the specific patient being treated. Typically, the amount employed will be the same dose as that used, if the given therapeutic agent is administered without IFN-γ.

EXAMPLES

Example 1

Inhibition of $PGF_{2\alpha}$-induced spreading response of adult myocytes by IFN-γ

Materials and Methods

Adult myocyte cultures The procedure used for the isolation of ventricular myocytes from adult rats was a modification of a procedure described by Piper *et al.*, *supra*, and is detailed in Lai *et al.*, *supra*. For each myocyte preparation, one male Sprague-Dawley rat weighing about 250 g was anesthetized with pentobarbital sodium, and the heart was removed. Extraneous tissue was trimmed from the heart, and it was mounted onto a Langendorff system that was temperature controlled at 37°C. The heart was perfused with about 40 ml of Krebs buffer (110 mM NaCl, 2.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4.7H_2O$, 25 mM $NaHCO_3$ and 11 mM glucose). A solution containing 30 mg of collagenase and 12.5 µl of 100 mM $CaCl_2$ in 50 ml of Krebs buffer was *then recirculated through the heart for 30 minutes. The heart was removed from the Langendorff apparatus, and* the atria and connective tissues were removed. The ventricles were cut into 2 mm cubes with dissecting scissors, and further digested in a fresh collagenase solution (30 mg collagenase and 400 mg BSA dissolved in Krebs buffer with 12.5 µl of 100 mM $CaCl_2$) for five minutes at 37 °C. During the digestion, the tissue suspension was gently hand shaken one time per minute. After the digestion, the supernatant was removed and saved, and the remaining tissue was further digested in fresh collagenase solution for an additional five minutes.

Isolated adult rat myocytes were plated on laminin-coated plates at a density of $3x10^3$ cells/ml. After 72 hours of appropriate stimulation, the cells were fixed with gluteraldehyde and stained with Eosin Y. Images of rod shaped cells were captured under fluorescent microscopy and maximum breadth was determined using imaging software (Simple 32, Compix Imaging, Mars, PA).

Results

IFN-γ inhibits the spreading of adult cardiac myocyte induced by the hypertrophy factors $PGF_{2\alpha}$ and phenylephrine

$PGF_{2\alpha}$ and the α-adrenergic agonist phenylephrine have been shown to induce hypertrophy of cultured neonatal rat cardiac myocytes (Adams *et al.*, J. Biol. Chem. 271:1179-1186 [1996]; Lai *et al.*, Am. J. Physiol. (Heart Circ. Physiol.) 271: H2197-H2208 [1996]; Meidell *et al.*, Am. J. Physiol. 251:H1076-H1084 [1986]; Simpson, J. Clin. Invest. 72:732-738 [1983]; Simpson, Circ. Res. 56:884-894 [1985]). Adult rat ventricular myocytes spread when exposed to these factors in culture (Lai *et al.*, *supra*; Piper *et al.*, "Adult ventricular rat heart muscle cells", in: Cell Culture Techniques in Heart and Vessel Research, H.M. Piper, Editor, 1990, Springer-Verlag: Berlin, p. 36-60.) Adult myocytes have a rod-like morphology. When these cells are exposed to 0.1 µM $PGF_{2\alpha}$, the rod shaped cells flatten and spread (Figure 1). The spreading response was quantified by measuring the maximum cell breadth of at least 200 rod shaped cells and plotting this value vs the percent frequency of cell breadth in the population. $PGF_{2\alpha}$ induced a significant change in the maximum cell breadth as evidenced by a shift in the population distribution for this parameter compared to control cells ($P<0.001$). Treating cells with IFN-γ significantly inhibited their response to $PGF_{2\alpha}$ ($P<0.001$ $PGF_{2\alpha}$ + IFN-γ compared to $PGF_{2\alpha}$). The inhibitor effect of IFN-γ on $PGF_{2\alpha}$-induced myocyte spreading was dose dependent (Figure 2) over a concentration range

that is consistent with the biological response to IFN-γ in cardiac myocytes and other cell systems (Singh *et al.*, J. Biol. Chem. 271: 1111-1117 [1996]; Pinsky *et al.*, J. Clin. Invest. 95:766-685 [1995]; Ungureanu-Longrois *et al.*, Circ. Res. 77: 494-502 [1995]; Soderberg-Naucler *et al.*, J. Clin. Invest. 100:3154-3163 [1997]; Gou *et al.*, J. Clin. Invest. 100:829-838 [1997]; Marra *et al.*, Can J. Cardiol. 12:1259-1267 [1996]). The ability to inhibit $PGF_{2\alpha}$ induced myocyte spreading appears to be specific to IFN-γ since several other cytokines including IL-1α, IL-1β, IL-2, IL-6, TNF-α, IFN-α, and IFN-β could not inhibit the spreading response. The inhibitory effect of IFN-γ is not specific for $PGF_{2\alpha}$. IFN-γ can also inhibit spreading induced by phenylephrine (Figure 3).

Example 2

Inhibition of cardiac hypertrophy *in vivo*

Materials and Methods

Animals All experimental procedures conformed to the guiding principles of the American Physiology Society, and were approved by Genentech's Institutional Animal Care and Use Committee. The animals used in this study were male Sprague/Dawley(SD) rats (8 weeks of age, Charles River Breeding Laboratories, Inc.). The animals were acclimated to the facility for at least one week before experiments, fed a pelleted rat chow and water *ad libitum*, and housed in a light and temperature controlled room.

Administration of fluprostenol and/or IFN-γ Rats received subcutaneous injection of fluprostenol (Cayman Chemical, Ann Arbor, MI) at 0.15 mg/kg, recombinant mouse IFN-γ (Genentech, Inc., South San Francisco, CA) at 0.08 mg/kg, combination of fluprostenol and IFN-γ, or saline vehicle, twice a day for 14 days. In the IFN-γ and fluprostenol+IFN-γ groups, animals were pretreated with IFN-γ for one day. Body weight was measured before and after treatment. A previous study has shown that the dose of fluprostenol used here is the lowest dose which produces a significant cardiac hypertrophy in rats. Lai *et al.*, *supra*. A pilot study demonstrated that IFN-γ at the dose indicated above inhibited fluprostenol-induced cardiac hypertrophy with little effects on body weight in rats.

Hemodynamic assessment Thirteen days after treatment, rats were anesthetized with intraperitoreal injection of ketamine 80 mg/kg (Aveco Co., Inc., Fort Dodge, Iowa) and xylazine 10 mg/kg (Rugby Laboratories, Inc., Rockville Center, NY.). A catheter (PE-10 fused with PE 50) filled with heparin-saline solution (50 U/ml) was implanted into the abdominal aorta, via the right femoral artery, for measurement of mean arterial pressure (MAP) and heart rate (HR). The catheter was exteriorized and fixed at the back of the neck.

One day after catheterization, the arterial catheter was connected to a Model CP10 pressure transducer (Century Technology Company, Inglewood, CA, USA) that was coupled to a Grass Model 7 polygraph (Grass Instruments, Quincy, MA, USA). MAP and HR were measured simultaneously in conscious, unrestrained rats.

Measurement of organ weights Under anesthesia with ketamine/xylazine, the heart, kidney, and spleen were removed, dissected and weighed. The left ventricle was stored at 80°C for evaluation of gene expression.

Animal model of pressure overload The induction of pressure overload by partial ligation of the abdominal aorta in rats was as described previously. Kimura *et al.*, Am. J. Physiol. 1989;256 (Heart Circ. Physiol. 25):H1006-H1-11; Batra *et al.*, J. Cardiovasc. Pharmacol. 17(suppl. 2), S151-S153 (1991). In brief, rats were anesthesized with ketamine/xylazine as described above. A 3 cm midline incision was made in the abdominal wall. The abdominal aorta between the diaphragm and the renal artery was exposed and looped with 5-0 silk suture. The

suture was tightened around a gauge 23 needle, and then the needle was withdrawn. Sham animals received the surgery without tightening the suture.

Experimental protocol in rats with pressure overload The rats with aortic banding randomly received subcutaneous injection of IFN-γ at 0.08 mg/k twice a day for one day before surgery and for 14 days after surgery. Sham animals were not treated. Thirteen days after treatment, a catheter was implanted into the right carotid artery under anesthesia as indicated above. One day after implantation, arterial pressure and HR were measured in conscious rats. The heart and other organs including the liver, kidney, and spleen were removed, weighed, and fixed in 10% buffered formalin for pathological studies. The left ventricle was quickly dissected and frozen with liquid nitrogen in some animals and stored at -80°C for assessment of gene expression.

Statistical analysis Results are expressed as mean±SEM. One way analysis of variance (ANOVA) was performed to assess differences in parameters between groups. Significant differences were then subjected to *post hoc* analysis using Newman-Keuls method; $P<0.05$ was considered significant.

RNA preparation Total RNA was isolated using RNeasy Maxi Columns (Qiagen) according to the manufacturer's instructions.

RT-PCR Real-time RT-PCR (TaqMan) technology was used to compare the gene expression between the various treatment groups. An oligonucleotide probe containing a fluorescent reporter dye, 6-carboxytetramethyl-rhodamine(TAMRA), at the 3'-end was designed to hybridize to the amplicon defined by two PCR primers. A 3'-blocking phosphate prevents extension of the probe. The reporter dye is released from the probe by the 5' exonuclease activity of Taq polymerase during the extension phase of PCR reaction. The resulting fluorescence is monitored in the reaction tube by the sequence detector and quantified without further manipulation, hence the term "real-time". The threshold cycle number (Ct), defined as the point where the reporter fluorescence reaches a value greater than 10 times the standard deviation of the baseline, is proportional to the amount of amplicon produced from the sample. Since the fluorescence is detected during the exponential phase of the amplification, none of the reaction components are limiting. In each experiment, a control is analyzed that lacks the RNA template to monitor for contamination, and another control is included where the RT step is omitted to eliminate amplification of possible contaminating DNA as the source of the signal. Reactions are optimized to give the greatest fluorescence signal and smallest Ct by titration of magnesium and primer concentrations, and the product is run on an agarose gel to verify the presence of a single band at the predicted molecular weight. In addition, the sequence of the amplicon is screened against Genbank to eliminate the possibility of overlap with closely related genes.

For each sample the mRNA for each target gene is determined using a standard curve as described below and then normalized to the amount of glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) in the sample (see below for specifics of this calculation). The relative abundance of each target gene to GAPDH can then be compared among treatment groups.

RT-PCR was performed on 1 ng of total RNA per reaction using the TaqMan Model 7700 Sequence Detector (ABI-Perkin Elmer) (Gibson *et al.*, Genome Res. 6, 995-1001 [1996]). Amplification reaction conditions (for 50 µl) were 1x TaqMan Buffer A, 200 µM dATP, dCTP, dGTP, and 400 µM dUTP, 10% glycerol, 6.5 mM $MgCl_2$, 50 U MuLV reverse transcriptase, 20 U RNase Inhibitor, 1.25 U AmpliTaq Gold, 100 nM forward and reverse primers, and 100 nM fluorogenic probe. RT-PCR reagents and glycerol were purchased from Perkin Elmer

and Sigma, respectively. Reactions were performed in MicoAmp Optical Tubes and Caps (ABI-Perkin Elmer). TaqMan primers and probes were designed according to guidelines determined by Perkin Elmer and synthesized at Genentech, Inc., except for those for rodent GAPDH which were a generous gift from Perkin Elmer. Reverse transcription was performed at 48 °C for 30 minutes followed by heat activation of AmpliTaq Gold at 95 °C for 10 minutes. Thermal cycling was at 95°C for 30 seconds and 60 ° for 1.5 minutes for 40 cycles.

Quantitation of the TaqMan results was performed as described by Heid *et al.*, Genome Res. 6:986-994 (1996), with modifications. Briefly, standard curves (1:5 serial dilution) for each target gene of interest were run in duplicate. The Ct was plotted on the Y axis vs the log of the total RNA concentration (X axis), and the equation describing the line was determined. MRNA for each target gene was determined from the appropriate standard curve by entering the Ct (Y value) and solving for the input mRNA (X). The value for the target gene was then normalized to GAPDH by solving the following equation: $10^{X1}/10^{X2}$, where X1 is the target gene, and X2 is GAPDH.

Results

IFN-γ inhibits cardiac hypertrophy in vivo

Chronic administration of fluprostenol, an agonist analog of $PGF_{2\alpha}$, has been shown to induce cardiac hypertrophy *in vivo*, and rats with pathologic cardiac hypertrophy induced by myocardial infarction have chronically elevated levels of extractable $PGF_{2\alpha}$ in their myocardium (Lai *et al., supra*). Thus, factors that can inhibit the effects of $PGF_{2\alpha}$ on myocardial growth *in vivo* may be useful for treating cardiac hypertrophy. Rats were dosed with fluprostenol in the presence and absence of IFN-γ for two weeks, and the effects on cardiac hypertrophy were determined. Absolute weight of the heart, ventricles, and left ventricle tended to increase in the fluprostenol-treated rats, compared to vehicle controls, and there was significant decrease in these parameters in rats treated with fluprostenol + IFN-γ relative to fluprostenol treated rats (Table 1). Treatment with fluprostenol resulted in a significant increase in the ratio of heart, ventricular, and left ventricular weights to body weight (BW), indicating that fluprostenol induced cardiac hypertrophy (Figure 4). IFN-γ inhibited fluprostenol induced hypertrophy. Rats receiving fluprostenol + IFN-γ had significantly decreased heart, ventricular and left ventricular weight, normalized by BW, compared to animals in the fluprostenol groups (Figure 4). Comparison between the IFN-γ and vehicle treated groups showed that administration of IFN-γ alone did not significantly alter absolute or BW-normalized heat, ventricular, or left ventricular weights (Table 1, Figure 4).

Chronic administration of fluprostenol was associated with a significant decline in mean arterial pressure (MAP) compared to vehicle treated controls (Figure 5). IFN-γ had no effect on MAP compared to vehicle, and did not affect the MAP of animals treated with fluprostenol. There was significant alteration in the heart rate in the four treatment groups (Figure 5). These results indicate that IFN-γ did not inhibit hypertrophy induced by fluprostenol by counteracting the hemodynamic effects of the treatment.

IFN-γ not only inhibited the increase in cardiac mass associated with fluprostenol administration, but also the alterations in cardiac gene expression associated with fluprostenol induced hypertrophy (Figure 6). There was an increase in the abundance of mRNA for α-skeletal actin, collagen I, and natriuretic factor in the hearts of rats treated with fluprostenol compared to vehicle. The mRNA for sarcoplasmic reticulum calcium ATPase was significantly reduced in these rats. IFN-γ inhibited all but the atrial natriuretic factor response.

IFN-γ was also tested in a rodent model of cardiac hypertrophy induced by pressure overload generated by abdominal aortic banding. Aortic constriction resulted in cardiac hypertrophy as evidenced by substantial increases in absolute heart, atrial, ventricular and left ventricular weights, and also the ratios of these weights to BW. Treatment with IFN-γ significantly attenuated cardiac hypertrophy in this model (Table 2 and Figure 7 and 8).

The effect of IFN-γ on other organs was also examined (Table 2). Neither aortic banding nor IFN-γ treatment altered kidney weight and the ratio of kidney weight to BW. Compared to sham-operated animals, liver weight and the ratio of BW tended to decrease in aortic-banded rats treated with vehicle, but not in those treated with IFN-γ. Aortic constriction caused a significant elevation in absolute and BW-normalized spleen weight, that was exaggerated by IFN-γ treatment. Thus, the effects of IFN-γ on cardiac mass were not due to a generalized effect on organ weight.

Mean arterial pressure, systolic pressure, and diastolic pressure were markedly higher in rats with aortic constriction compared to sham-operated controls, and the incremental increase in arterial pressure was not different between banded rats treated with IFN-γ or vehicle (Figure 9). This result indicates that the attenuation of cardiac hypertrophy observed in banded rats receiving IFN-γ did not relate to an alteration in afterload.

Aortic constriction resulted in several changes in cardiac gene expression. The relative abundance of mRNA for β-myosin heavy chain, α-smooth muscle and α-skeletal actins, atrial natriuretic factor, collagens I and III, and fibronectin were all increased in banded rats compared to sham-operated controls. The effects on two of these genes; α-smooth muscle actin and collagen I were inhibited by IFN-γ (Table 3).

Taken together, the results in Examples 1 and 2 show that IFN-γ can inhibit cardia hypertrophy. The effects of IFN-γ are not limited to inhibiting an increase in cardiac mass induced by hypertrophic stimuli, IFN-γ can also inhibit certain of the molecular alterations that occur in the hypertrophied heart at the level of gene expression. It is especially noteworthy that IFN-γ inhibited the induction of collagen I gene expression *in vivo*, both in response to chronic stimulation with fluprostenol and in a model of hypertrophy induced by pressure overload. Collagen I accounts for approximately 75% of myocardial collagen (Ju *et al.*, Can. J. Caridol. 12:1259-1267 [1996]). *Increased extracellular matrix deposition and interstitial fibrosis that accompany cardiac hypertrophy can* contribute to the pathophysiology of heart failure. By inhibiting collagen I production, IFN-γ may reduce interstitial fibrosis in the setting of heart failure.

Table 1. Body Weight and Organ Weight in Rats Treated with Flup and/or IFN

	Vehicle	Flup	Flup+IFN	IFN
BWO (g)	292.4±1.7	292.3±2.2	292.8±2.1	292.5±3.2
BW (g)	391.6±6.3	381.1±4.4	377.6±4.5	380.8±6.1
ΔBW (g)	99.2±5.5	91.9±4.0	84.8±3.9	88.3±5.0
HW (g)	.966±.022	1.000±.018	.9279±.016#	.956±.029
VW (g)	.922±.022	.957±.017	.889±.015#	.914±.029
LVW (g)	.706±.018	.740±.013	.678±.011##	.696±.023
KW (g)	1.440±.035	1.397±.038	1.377±.031	1.327±.035*

KW/BW (g/kg)	3.678±.075	3.632±.076	3.648±.070	3.483±.069
SW (g)	.799±.050	.880±.048	1.009±.042*	.924±.068
SW/BW (g/kg)	2.065±.149	2.309±.130	2.676±.082**	2.415±.149

Data expressed as mean±SEM, and animal numbers are 14, 14, 14, and 9 in the Vehicle, Flup, Flup+IFN, and IFN group, respectively. Vehicle, saline; Flup, fluprostenol; IFN, interferon γ, BWO, basal levels of body weight; BW, body weight post treatment; ΔBW, BW-BWO; HW, heart weight; VW, ventricular weight; LVW, left ventricular weight; KW, kidney weight; SW, spleen weight. * p<0.05, ** P<0.01, compared to the Vehicle group, # p<0.05, ## p<0.01, compared to the Flup group.

Table 2. Body Weight, Organ Weight, and HR in Rats with Pressure Overload

	Sham	PO+vehicle	PO+IFN
BWO (g)	278.8±1.9	279.4±1.3	279.0±1.3
BW (g)	367.9±6.8	347.5±5.7*	355.5±5.2
ΔBW (g)	89.1±5.8	68.1±5.6*	76.5±4.7
AW (g)	.038±.002	.056±.002**	.046±.003*##
AW/BW (g/kg)	.104±.004	.162±.006**	.129±.007*##
KW (g)	1.438±.051	1.334±.033	1.349±.071
KWBW (g/kg)	3.894±.078	3.841±.070	3.776±.071
LW (g)	13.84±.55	12.53±.36	13.96±.47#
LW/BW (g/kg)	37.46±.96	36.01±.71	38.94±.92#
SW (g)	.724±.030	.839±.026*	1.170±.053**##
SW/BW (g/kg)	1.959±.051	2.418±.069**	3.261±.121**##
HR (bpm)	371±12	415±12*	418±19*

Data expressed as mean±SEM. Animal numbers are 16, 22, and 21 in the Sham, PO+vehicle, and PO+IFN group, respectively, for all parameters except HR for which animal number are 7, 8, and 7, respectively. PO, pressure overload; IFN, interferon γ; BWO, basal levels of body weight; BW, body weight post treatment; ΔBW, BW-BWO; AW, atrial weight; KW, kidney weight; LW, liver weight; SW, spleen weight; HR, heart rate. *p<0.05, ** P<0.01, compared to the sham group. # p<0.05, ## p<0.01, compared to the PO+vehicle group.

Table 3. Effect of IFN on Gene Expression

Treatment	SHAM + Vehicle	PO + Vehicle	PO + IFN
ANF	0.98±0.37	5.29±1.21†	3.61±1.32
βMHC	0.89±0.24	1.91±0.15†	1.71±0.14†
SKA	0.95±0.13	3.35±0.46†	2.47±0.51†
SMA	0.71±0.06	0.89±0.04†	0.77±0.06
COLI	0.55±0.05	0.91±0.09†	0.77±0.10
COLIII	0.44±0.05	0.66±0.08†	0.72±0.09†
FIB	0.66±0.17	1.03±0.11†	0.97±012

PO indicates pressure overload; IFN, interferon-gamma; ANF, atrial natriuretic factor; βMHC, β-myosin heavy chain; SKA, α-skeletal actin; SMA, α-smooth muscle actin; COLI, collagen I; COLIII, collagen III; FIB, fibronectin. Expression levels are calculated as ratios to glyceraldehyde-3-phosphate dehydrogenase. n=6 per group Values are mean±SEM. †P<0.05 vs sham+vehicle group.

†

Claims:

1. A method of treating cardiac hypertrophy comprising administering to a patient having cardiac hypertrophy a therapeutically effective amount of interferon gamma (IFN-γ).

2. The method of claim 1 wherein said patient is human.

3. The method of claim 2 wherein said IFN-γ is recombinant human IFN-γ(rh-IFN-γ).

4. The method of claim 3 wherein said IFN-γ is rhIFN-γ-1b.

5. The method of claim 3 wherein said cardiac hypertrophy is characterized by the presence of an elevated level of $PGF_{2\alpha}$.

6. The method of claim 2 wherein said cardiac hypertrophy has been induced by myocardial infarction.

7. The method of claim 6 wherein said IFN-γ administration is initiated within 48 hours following myocardial infarction.

8. The method of claim 7 wherein said IFN-γ administration is initiated within 24 hours following myocardial infarction.

9. The method of claim 2 wherein said patient is at risk of developing cardiac hypertrophy.

10. The method of claim 9 wherein said patient has suffered myocardial infarction.

11. The method of claim 10 wherein said IFN-γ administration is initiated within 48 hours following myocardial infarction.

12. The method of claim 11 wherein said IFN-γ administration is initiated within 24 hours following myocardial infarction.

13. The method of claim 2 wherein said IFN-γ is administered in combination with at least one further therapeutic agent used for the treatment of cardiac hypertrophy or a heart disease resulting in cardiac hypertrophy.

14. The method of claim 13 wherein said further therapeutic agent is selected from the group consisting of β-adrenergic-blocking agents, verapamil, difedipine, and diltiazem.

15. The method of claim 14 wherein said β-adrenergic blocking agent is carvedilol, propranolol, metoprolol, timolol, oxprenolol or tertatolol.

16. The method of claim 13 wherein said IFN-γ is administered in combination with an antihypertensive drug.

17. The method of claim 13 wherein said IFN-γ is administered with an ACE-inhibitor.

18. The method of claim 13 wherein said IFN-γ is administered with an endosthelin receptor antagonist.

19. The method of claim 13 wherein said IFN-γ is administered following the administration of a thrombolytic agent.

20. The method of claim 18 wherein said thrombolytic agent is recombinant human tissue plasminogen activator (rht-PA).

21. The method of claim 13 wherein said IFN-γ is administered following primary angioplasty for the treatment of acute myocardial infarction.

22. A method for making a pharmaceutical composition for the treatment of cardiac hypertrophy, comprising admixing a therapeutically effective amount of interferon gamma (IFN-γ) with a pharmaceutically acceptable carrier.

23. The method of claim 21 wherein said pharmaceutical composition is liquid.

24. The method of claim 22 wherein said pharmaceutical composition comprises a preservative.

25. The method of claim 23 wherein said pharmaceutical composition is an injectable formulation.

26. A pharmaceutical product comprising:

(a) a pharmaceutical composition comprising at least one therapeutically effective dosage of IFN-γ;

(b) a container containing said pharmaceutical composition; and

(c) a label affixed to said container, or a package insert included in said pharmaceutical product referring to the use of said IFN-γ in the treatment of cardiac hypertrophy.

27. The pharmaceutical product of claim 25, wherein said container has a sterile access port.

28. The pharmaceutical product of claim 26 wherein said container is an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

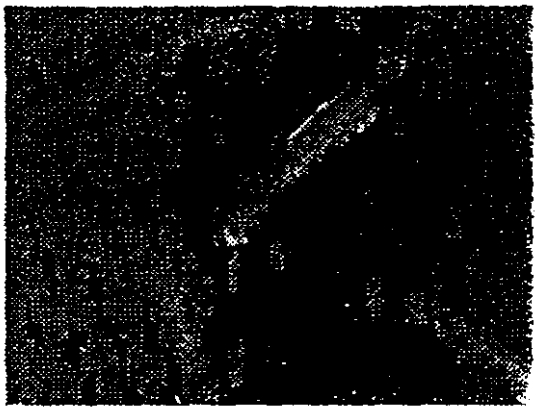


FIG. 1A

FIG. 1B

FIG. 1C

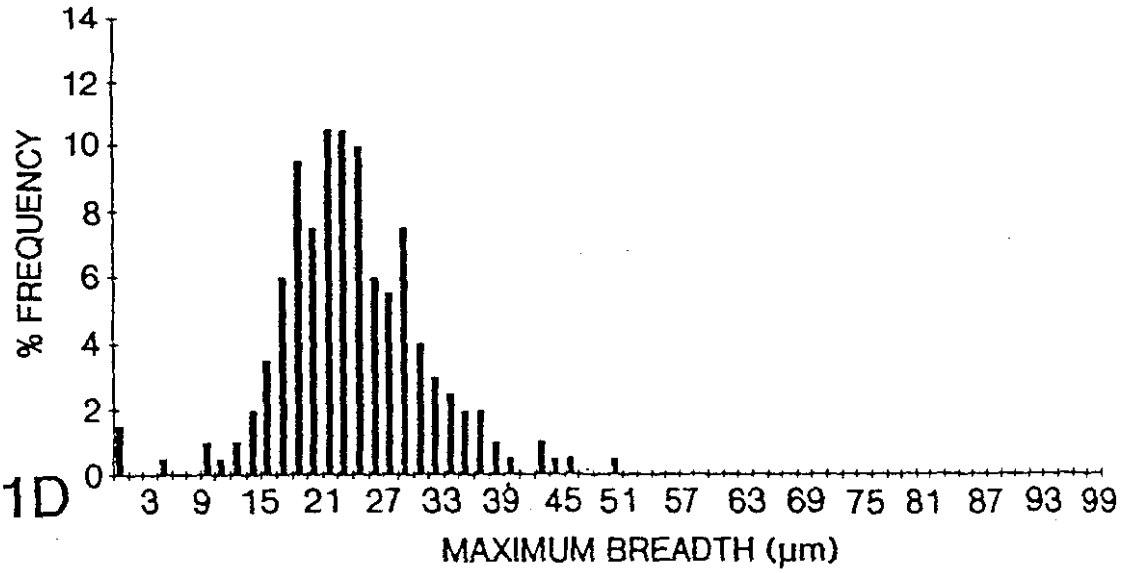


FIG. 1D

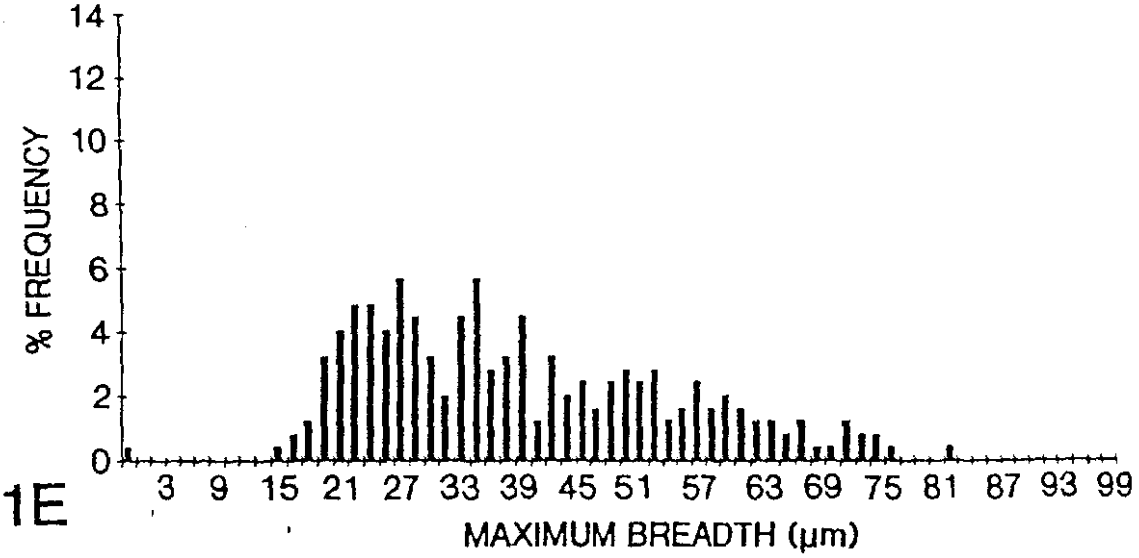


FIG. 1E

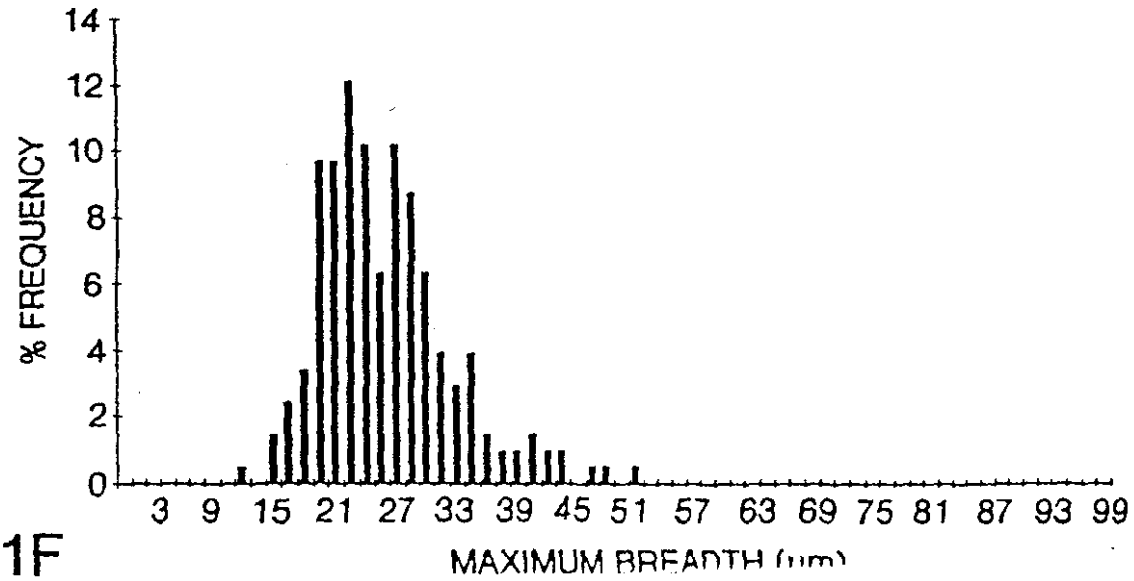


FIG. 1F

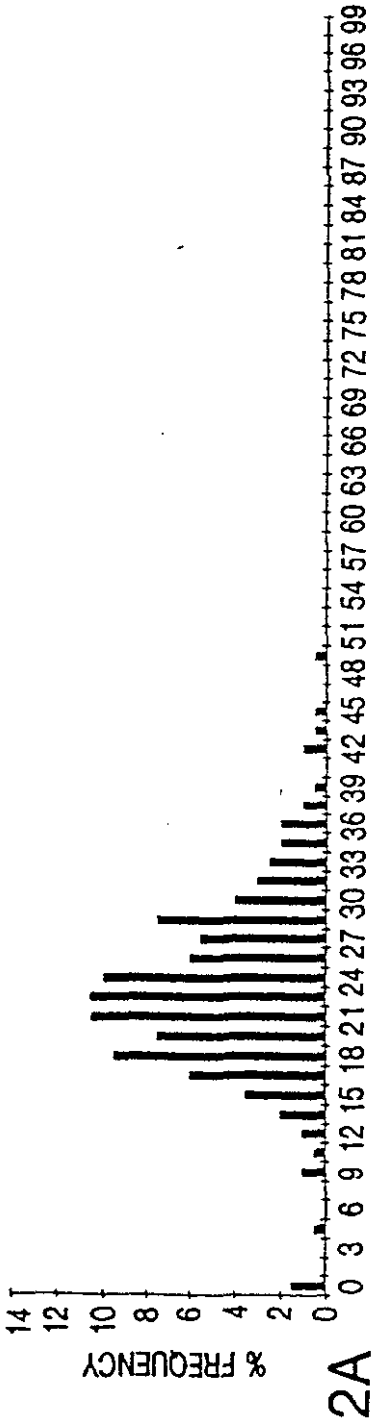


FIG. 2A

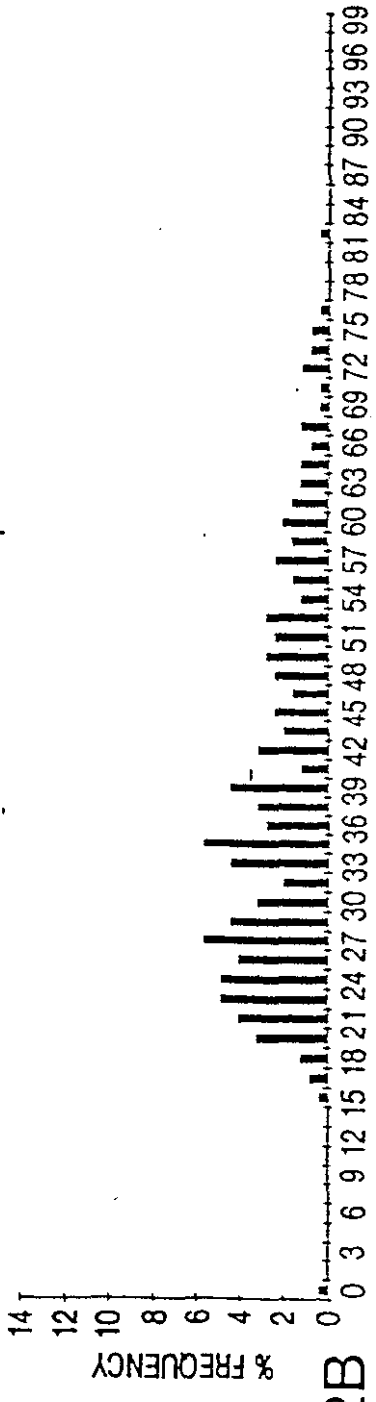


FIG. 2B

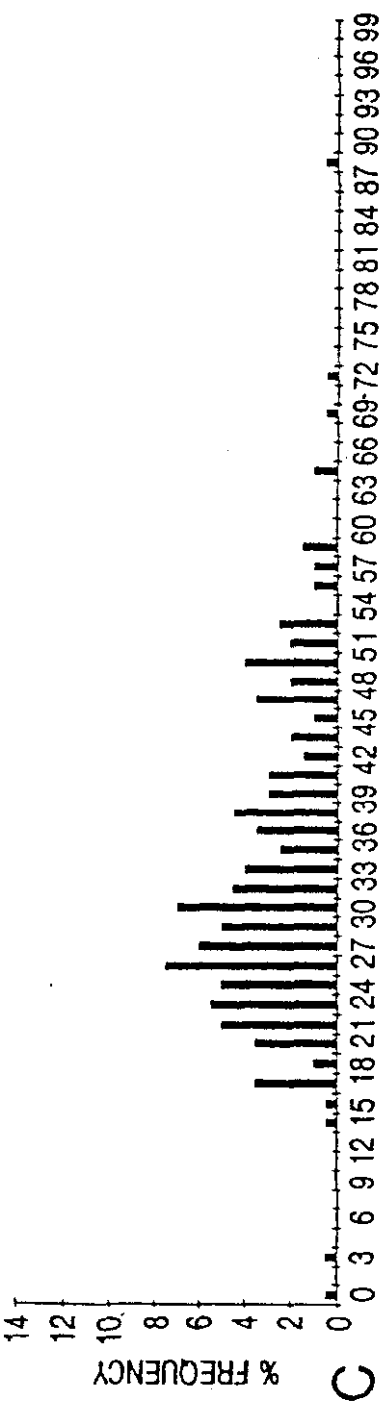
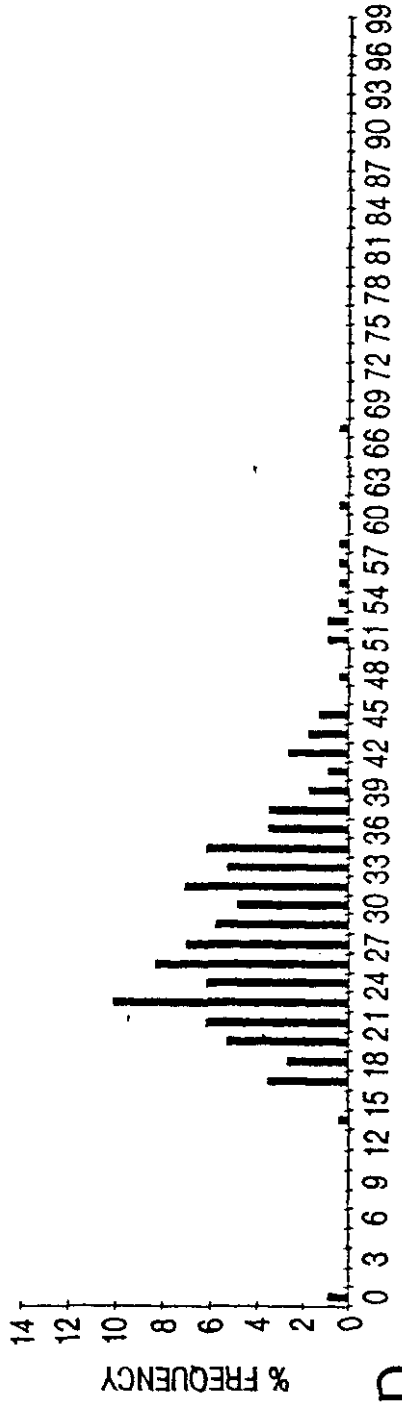


FIG. 2C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0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99
MAXIMUM BREADTH (µm)

FIG. 2D

% FREQUENCY
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0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99
MAXIMUM BREADTH (µm)

FIG. 2E

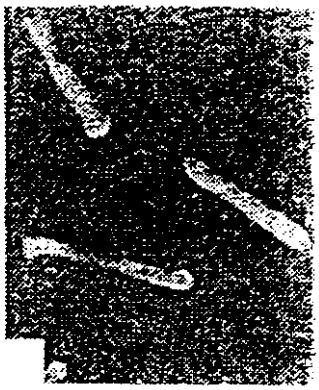

FIG. 3A

FIG. 3B

FIG. 3C

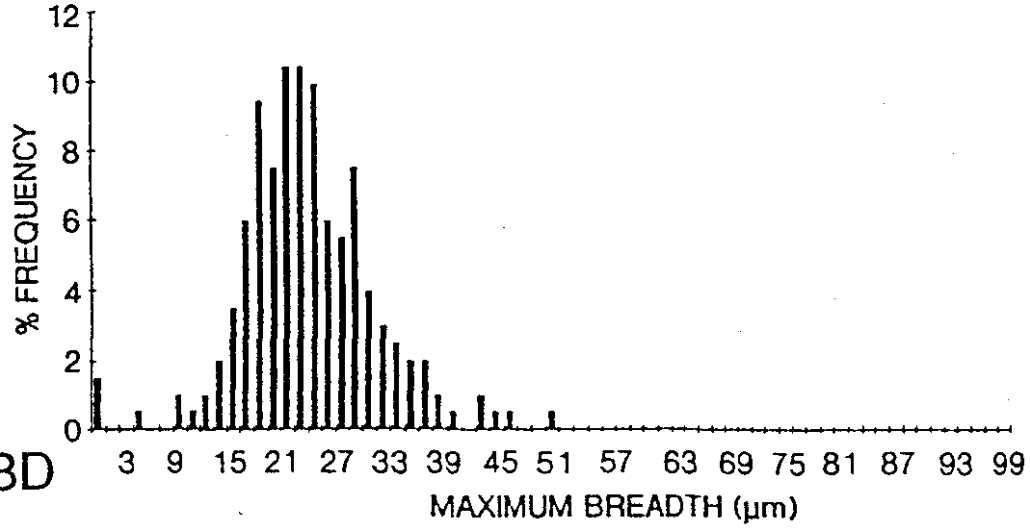

FIG. 3D

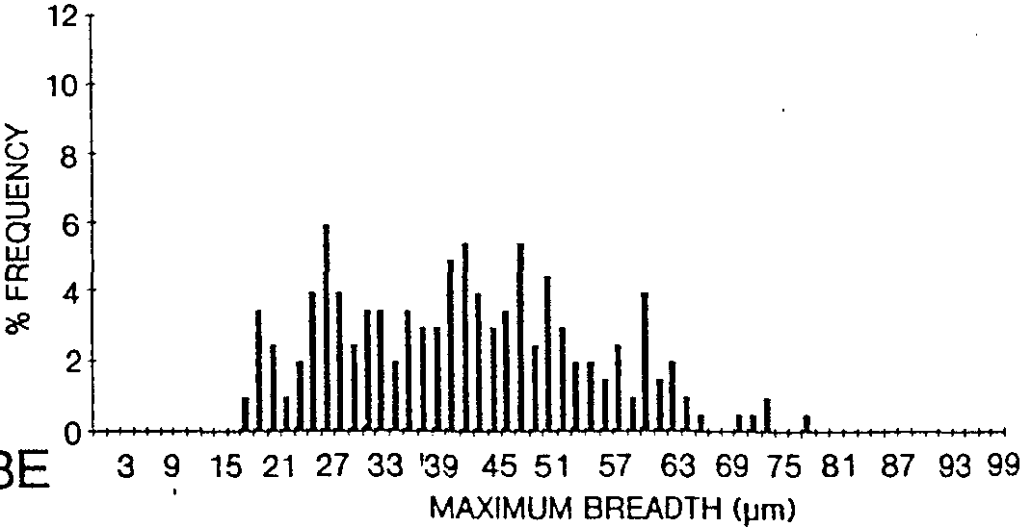

FIG. 3E

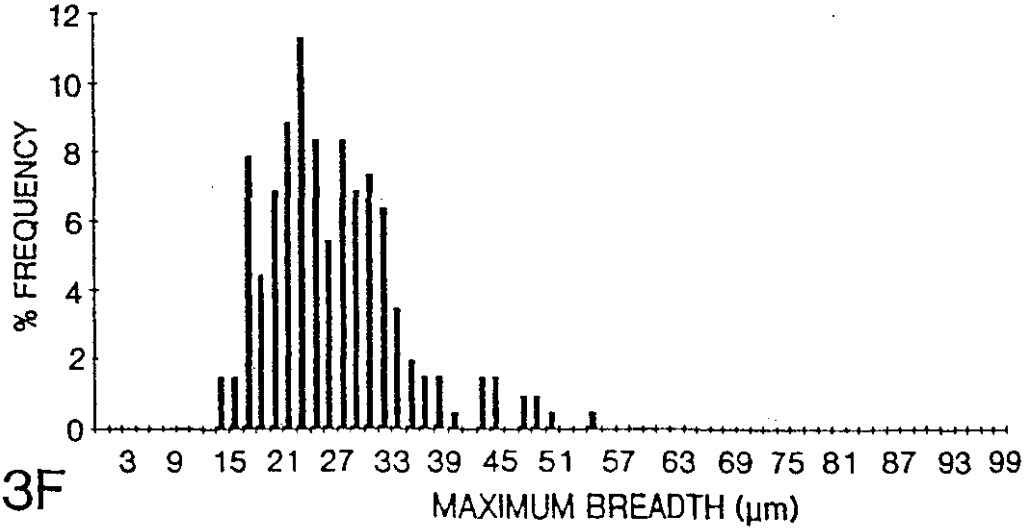

FIG. 3F

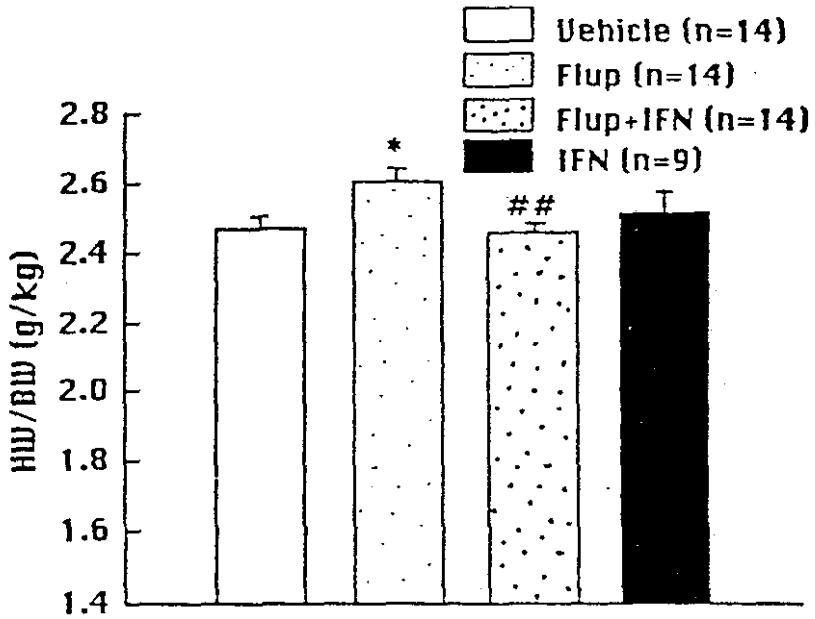

FIG. 4A

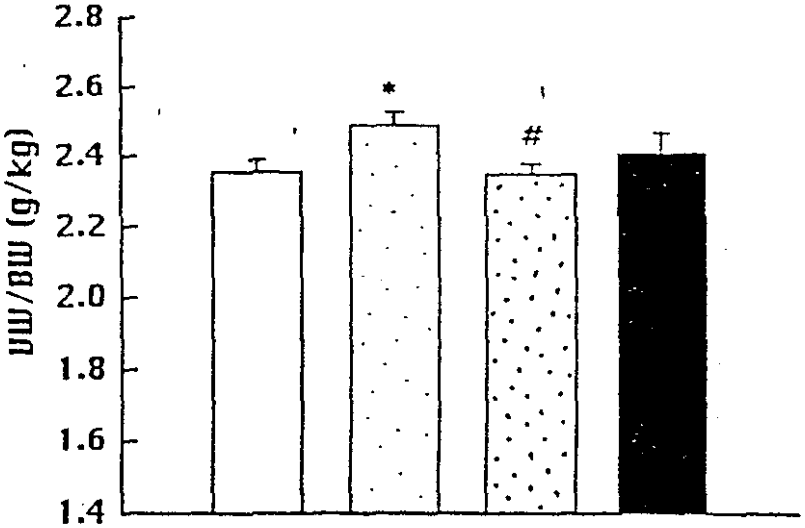

FIG. 4B

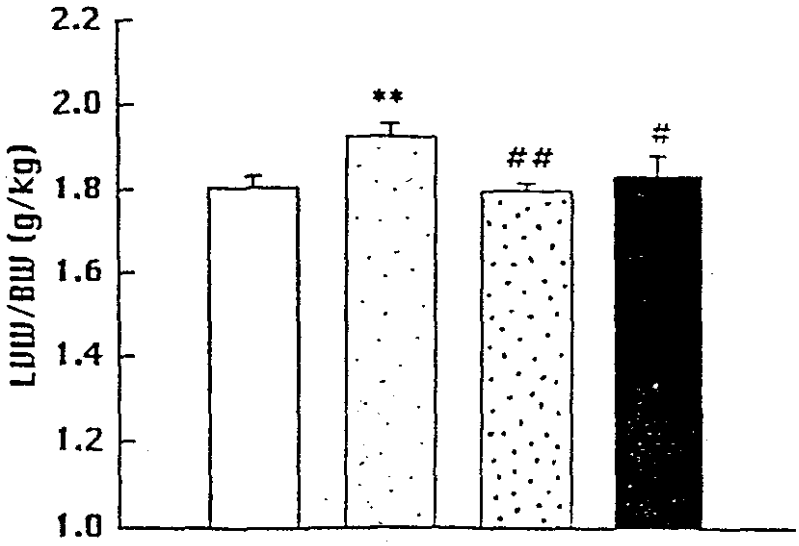

FIG. 4C

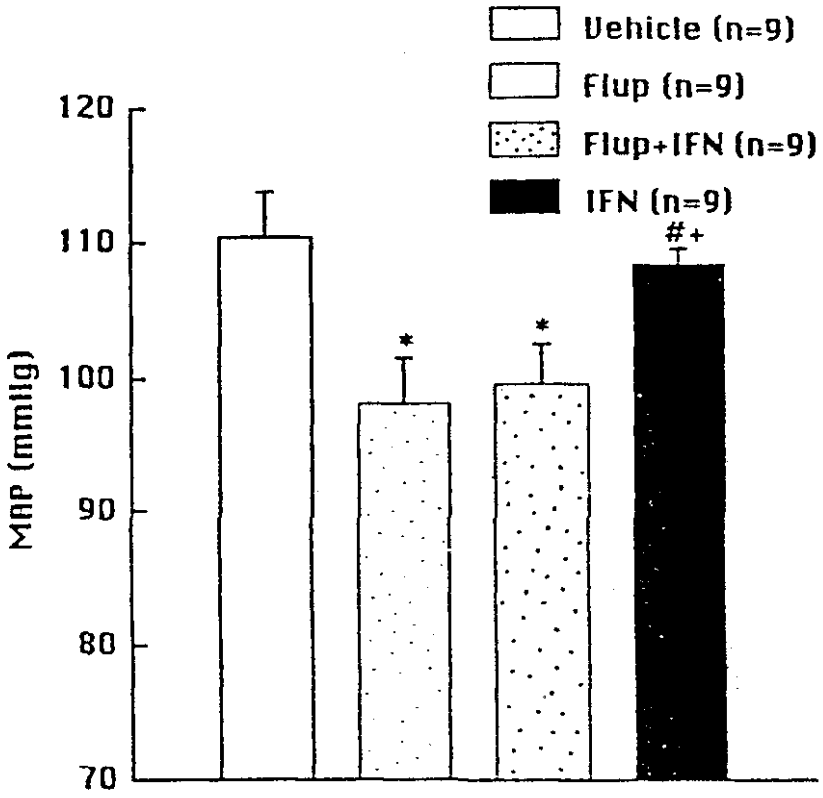
Vehicle (n=9)
Flup (n=9)
Flup+IFN (n=9)
IFN (n=9)
MAP (mmHg)
120
110
100
90
80
70
*
*
#+

FIG. 5A

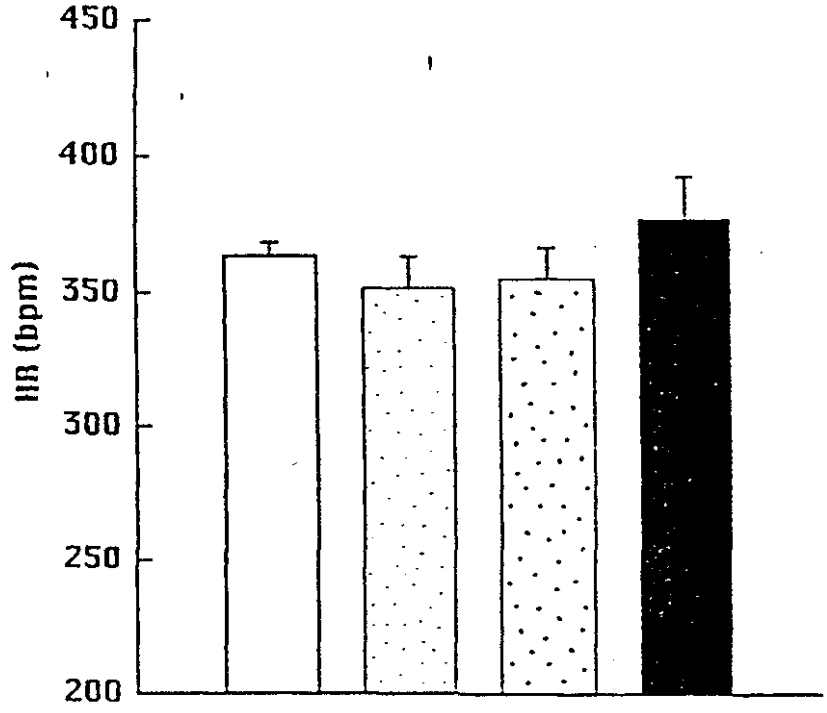
HR (bpm)
450
400
350
300
250
200

FIG. 5B

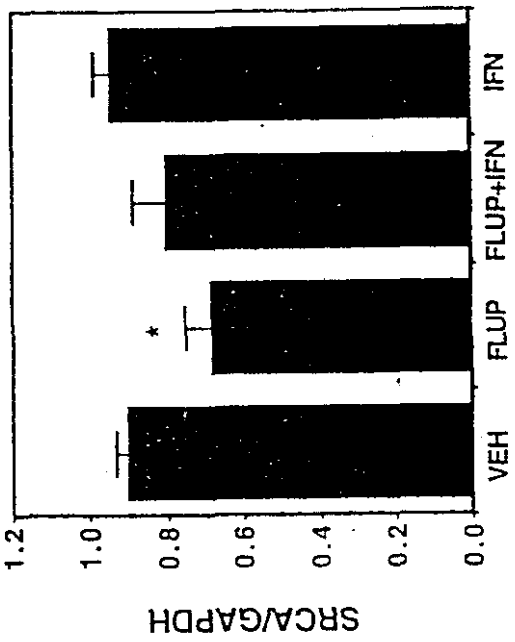

FIG. 6B

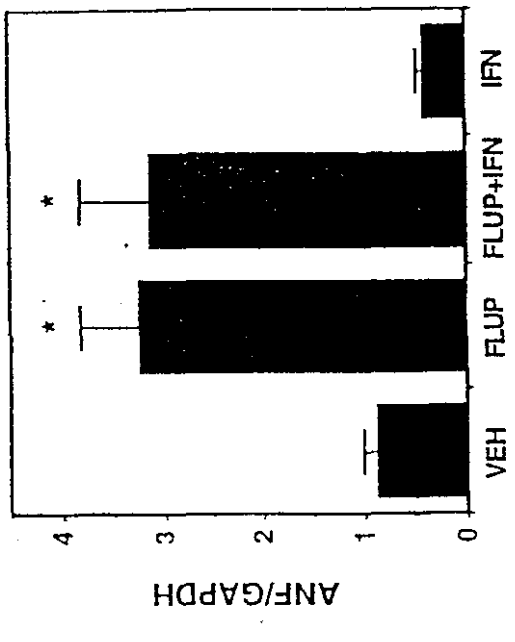

FIG. 6D

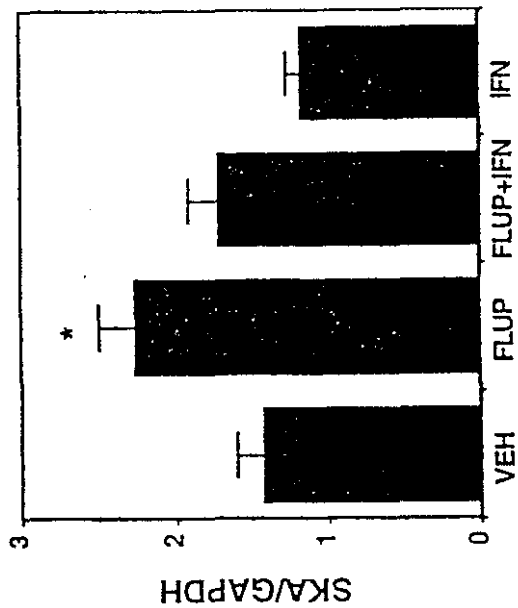

FIG. 6A

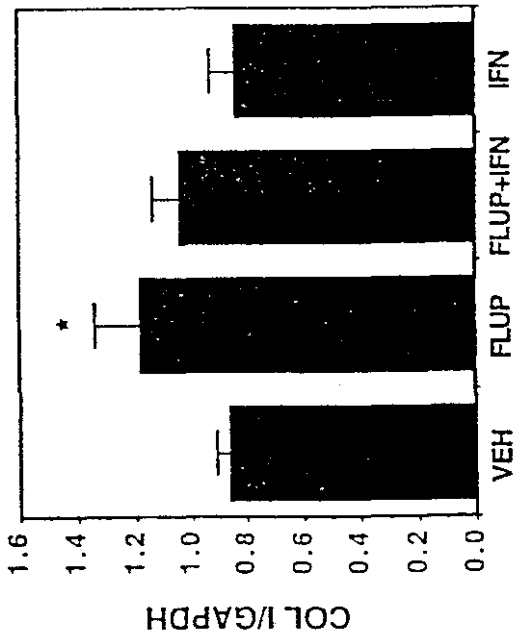

FIG. 6C

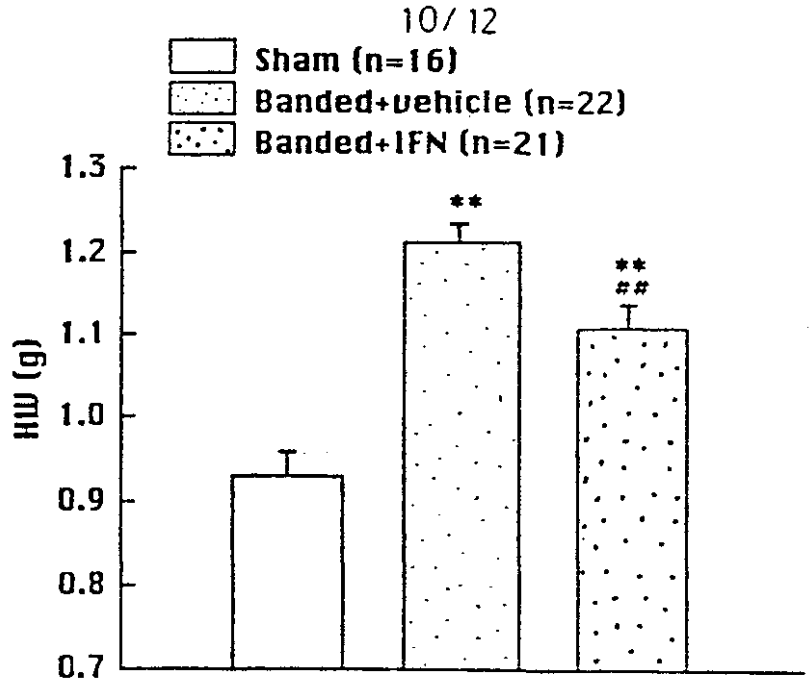

FIG. 7A

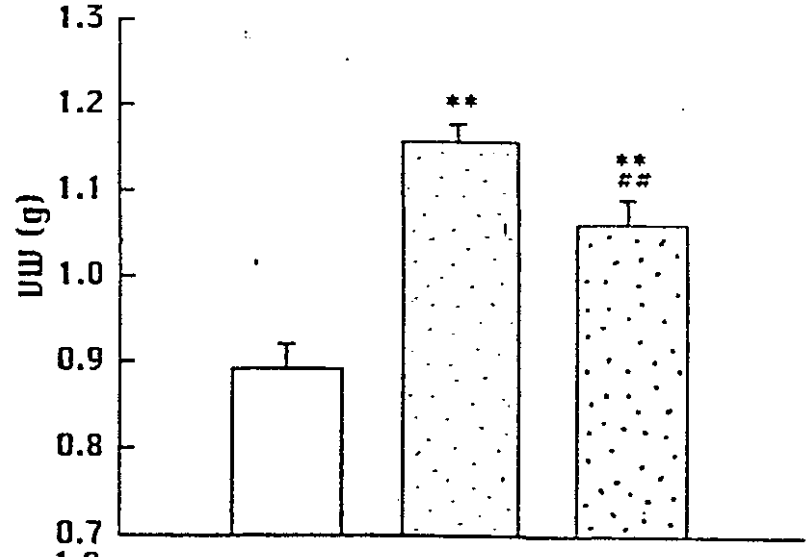

FIG. 7B

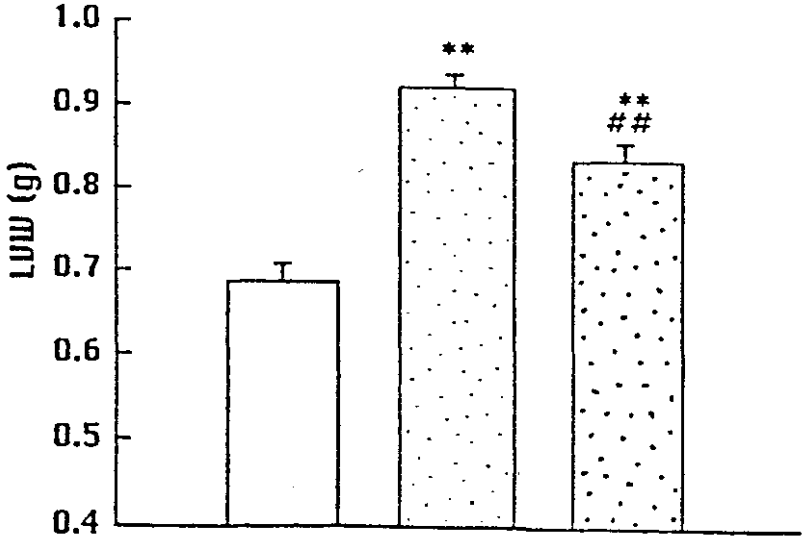

FIG. 7C

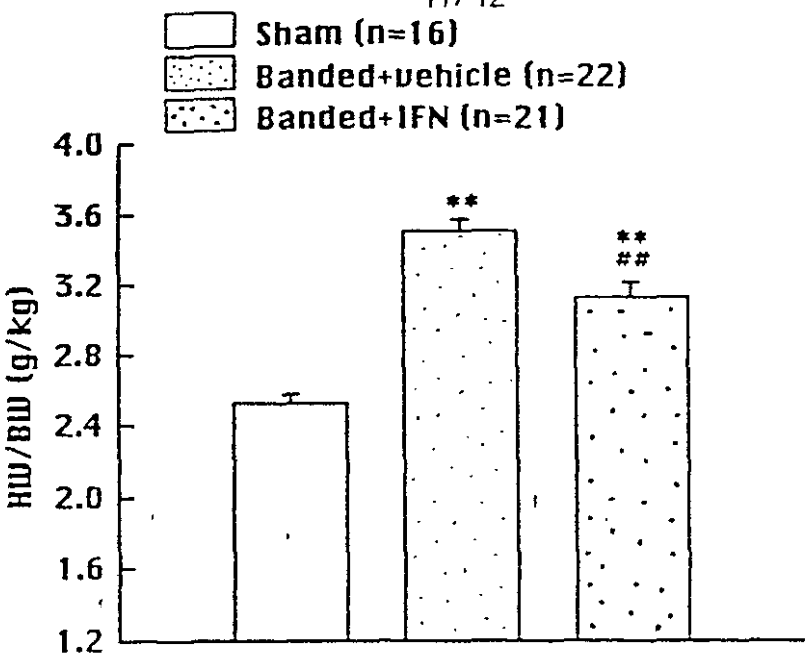

FIG. 8A

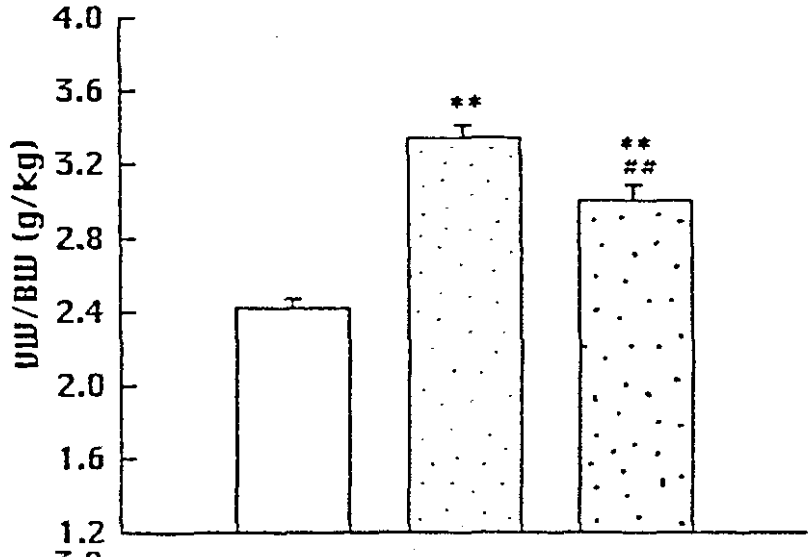

FIG. 8B

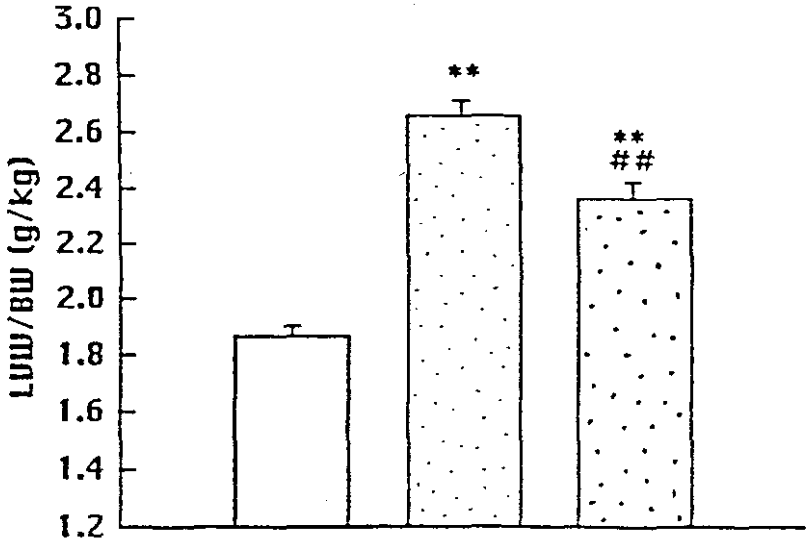

FIG. 8C

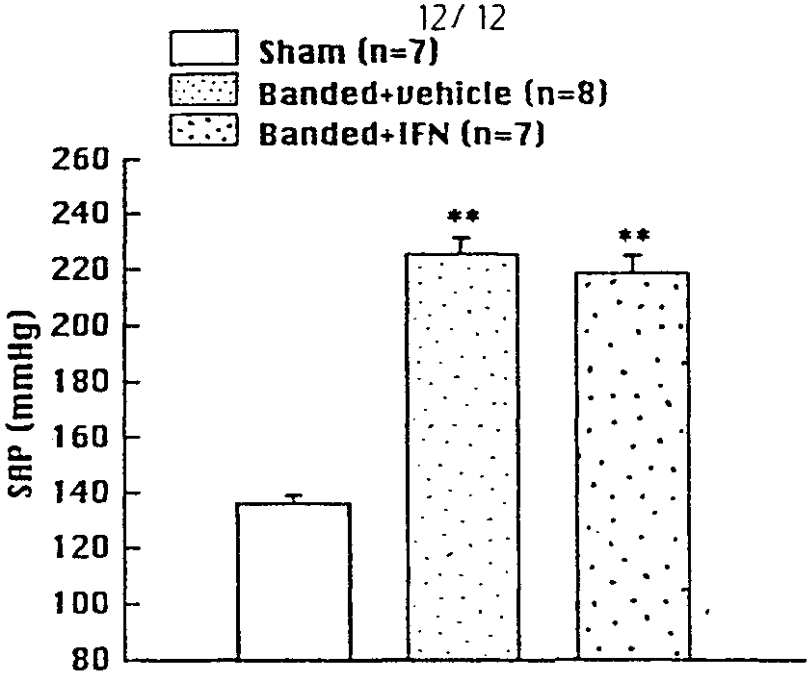

FIG. 9A

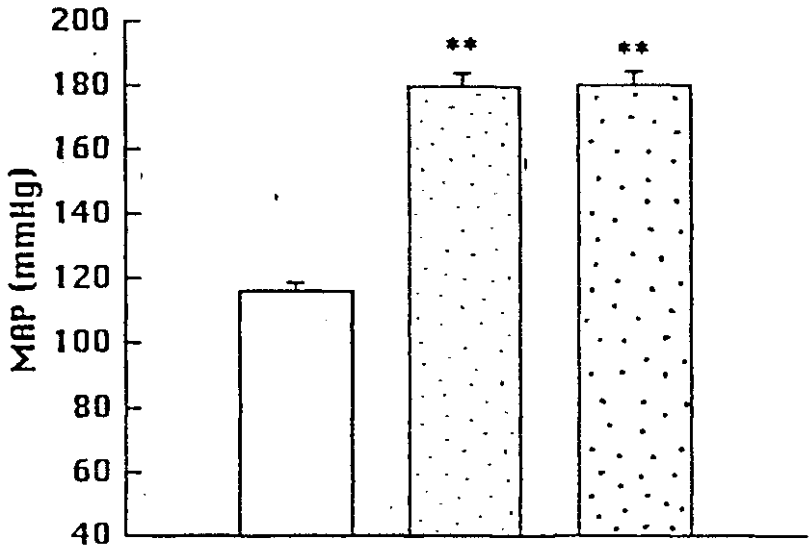

FIG. 9B

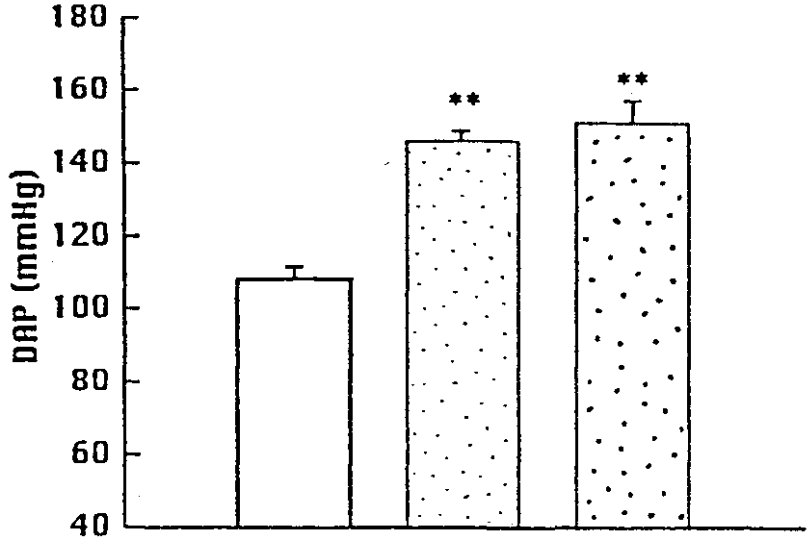

FIG. 9C

[19]中华人民共和国国家知识产权局

[51]Int. Cl[7]
A61K 38/21
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(A61K38/21,31: 55)
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(A61K38/21,31: 135)
(A61K38/21,31: 535)

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权利要求书2页 说明书23页 附图页数12页

[54]发明名称 心脏肥大的治疗方法

[57]摘要

本发明涉及用γ干扰素(IFN-γ)治疗心脏肥大的方法。许多病理情况，包括心肌梗塞、高血压、肥厚性心肌病和瓣膜返流，会导致心脏肥大。本发明治疗方法覆盖对心肌或有或没有结构损伤的心脏肥大的各个发展阶段，不论潜在的是何心脏疾病。

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权 利 要 求 书

1．一种治疗心脏肥大的方法，包括：给予心脏肥大患者治疗有效量的γ干扰素。

2．根据权利要求 1 所述的方法，其中所述的患者是人。

3．根据权利要求 2 所述的方法，其中所述的 IFN-γ是重组人 IFN-γ(rh-IFN-γ)。

4．根据权利要求 3 所述的方法，其中所述的 IFN-γ是 rh-IFN-γ-1b。

5．根据权利要求 3 所述的方法，其中所述的心脏肥大的特征是 $PGF_{2\alpha}$水平提高。

6．根据权利要求 2 所述的方法，其中所述心脏肥大是心肌梗塞诱导的。

7．根据权利要求 6 所述的方法，其中所述的 IFN-γ给予于心肌梗塞后 48 小时内开始。

8．根据权利要求 7 所述的方法，其中所述的 IFN-γ给予于心肌梗塞后 24 小时内开始。

9．根据权利要求 2 所述的方法，其中所述的患者具有患心脏肥大的危险性。

10．根据权利要求 9 所述的方法，其中所述的患者发生过心肌梗塞。

11．根据权利要求 10 所述的方法，其中所述的 IFN-γ给予于心肌梗塞后 48 小时内开始。

12．根据权利要求 11 所述的方法，其中所述的 IFN-γ给予于心肌梗塞后 24 小时内开始。

13．根据权利要求 2 所述的方法，其中所述的 IFN-γ与至少一种其它治疗心脏肥大或造成心脏肥大的心脏病的药物联用。

14．根据权利要求 13 所述的方法，其中所述的其它药物选自：β-肾上腺素能阻断剂、维拉帕米、difedipine 和地尔硫䓬。

15．根据权利要求 14 所述的方法，其中所述的β-肾上腺素能阻断剂是卡维地洛、普奈洛尔、美托洛尔、噻吗洛尔、氧烯洛尔或特他洛尔。

16．根据权利要求 13 所述的方法，其中所述的 IFN-γ与抗高血压药联用。

17．根据权利要求 13 所述的方法，其中所述的 IFN-γ与 ACE 抑制剂联用。

18．根据权利要求 13 所述的方法，其中所述的 IFN-γ与内皮肽受体拮抗剂联用。

19．根据权利要求 13 所述的方法，其中所述的 IFN-γ在溶血栓药之后使用。

20．根据权利要求 18 所述的方法，其中所述的溶血栓药是重组人组织血纤蛋白溶酶原活化剂(rht-PA)。

21. 根据权利要求 13 所述的方法，其中所述的 IFN-γ在进行主血管成形术治疗急性心肌梗塞后使用。

22. 一种制造治疗心脏肥大的药物组合物的方法，包括将治疗有效量的 IFN-γ与药学上认可的载体混合。

23. 根据权利要求 21 所述的方法，其中所述的药物组合物是液体。

24. 根据权利要求 22 所述的方法，其中所述的药物组合物含防腐剂。

25. 根据权利要求 23 所述的方法，其中所述的药物组合物是注射剂。

26. 一种药物产品，它包含：

(a)含至少一种治疗有效量的 IFN-γ的药物组合物；

(b)装所述药物组合物的容器；

(c)贴在所述容器上的标签，或包含在所述药物产品中的包装说明，说明所述 IFN-γ在治疗心脏肥大中的使用。

27. 根据权利要求 25 所述的药物产品，其中所述的容器具有无菌接口。

28. 根据权利要求 26 所述的药物产品，其中所述的容器是静脉输液袋或塞子可被皮下注射针穿通的管形瓶。

说　明　书

心脏肥大的治疗方法

发明领域

本发明主要涉及γ干扰素对心脏肥大的作用。更具体地说，本发明涉及用γ干扰素预防或治疗心脏肥大及相关病症。

发明背景

γ干扰素(IFN-γ)

干扰素是受到病毒或某些其它物质侵袭的细胞分泌的较小的单链糖蛋白。目前，干扰素主要分三类：白细胞干扰素(干扰素-α，α干扰素，IFN-α)，成纤维细胞干扰素(干扰素β，β干扰素，IFN-β)和免疫干扰素(干扰素-γ，γ干扰素，IFN-γ)。在对病毒感染的应答中，淋巴细胞主要合成α干扰素(另有少量与之不同的一种干扰素，一般称之为ω干扰素)，对成纤维细胞的感染则通常诱导产生β干扰素。α干扰素与β干扰素具有约 20-30%的氨基酸序列同源性。人 IFN-β的基因没有内含子，编码的蛋白与人 IFN-αI 具有 29%的氨基酸序列同源性，这说明 IFN-α与 IFN-β来自同一祖先(Taniguchi 等，Nature 285，547-549(1980))。相反，IFN-γ不是由病毒感染诱导而是由淋巴细胞应答促分裂原而合成的，而且在氨基酸序列上与另两类干扰素没有什么关系。已知α干扰素与β干扰素诱导 MHC I 类抗原，IFN-γ则诱导 MHC II 类抗原的表达并增强靶细胞呈递与 MHC I 类分子结合的病毒肽供细胞毒 T 细胞识别的效果。

IFN-γ是干扰素家族中的一员，具有α和β干扰素(IFN-α和β)抗病毒和抗增殖的特性，但与它们的不同的是对 PH2 不稳定。IFN-γ原本由促分裂原诱导淋巴细胞而产生。Gray, Goeddel 与其同事最早报道了重组生产人 IFN-γ (Gray 等，Nature 295，503-508[1982])，这也是美国专利 4,762,791，4,929,544，4,727,138，4,925,793，4,855,238，5,582,824，5,096,705，5,574,137 和 5,595,888 的主题。Gray 和 Goeddel 在大肠杆菌中产生的重组人 IFN-γ有 146 个氨基酸，该分子 N 末端的开始为 CysTyrCys。后来发现，天然人 IFN-γ(即，促分裂原诱导人外周血淋巴细胞产生并纯化得到的)多肽没有 Gray 等(同上)所述的 CysTyrCys N 末端。最近，测定了大肠杆菌产生的重组人 IFN-γ(rhIFN-γ)的晶体结构(Ealick 等，Science 252, 698-702[1991])，显示该蛋白是一种相互紧密缠绕的非共价二聚体，其中的两条多肽

链相互反平行。

已知 IFN-γ具有许多生物活性，包括抗肿瘤、抗菌和免疫调节活性。一种形式的重组人 IFN-γ(rhIFN-γ-1b，Actimmune®，Genentech, Inc. South San Francisco, Califonia)是市售的一种用于治疗慢性肉芽肿病的免疫调节药，该病的特征是吞噬细胞功能失常引起的皮肤、淋巴结、肝、肺和骨严重、反复感染(Baehner, R.L., Pediatric Pathol. 10，143-153(1990))。还有人提出用 IFN-γ治疗变应性皮炎，一种常见皮肤炎症，表现为严重瘙痒，病程迁延反复，间或恶化，独特的皮肤损伤临床形态和分布(参见 PCT 申请，WO91/07984，公开于 1991 年 6 月 13 日)，血管狭窄，包括治疗血管成形术和/或血管手术后的再狭窄(PCT 申请 WO90/03189，公开于 1990 年 4 月 5 日)，各种肺病，包括呼吸困难综合征(RDS)，例如成人呼吸困难综合征(ARDS)和新生儿型，又称自发性 RDS 或肺透明膜病(PCT 申请 WO89/01341，公开于 1989 年 2 月 23 日)。此外，已有人提出用 IFN-γ治疗各种变态反应，例如哮喘和 HIV 感染的相关疾病，例如机会性感染，如卡氏肺囊虫性肺炎和创伤性败血症。已发现，多发性硬化症患者的 IFN-γ产生受损，而且，据报道，在促分裂原刺激的 AIDS 患者单核细胞悬浮液中，IFN-γ产生受到严重抑制。有关论述可参见例如第 16 章“干扰素在疾病中可能具有的致病作用”，Interferons and Regulation Cytokines, Edward de Maeyer(1988, John Wilet and Sons Publishers)。

目前认为，IFN-γ，以及其它细胞因子，是可诱导氧化氮(iNOS)的诱导物，因此被描述成造成心力衰竭的潜在致炎机制、心脏对败血症或移植排斥的应答、以及各种病因的扩张性心肌病发展的重要介导物。Ungureanu-Longrois 等，Circ. Res. 77, 494-502(1995); Pinsky 等，J. Clin. Invest. 95, 677-685(1995); Singh 等，J. Biol. Chem. 270, 28471-8(1995); Birks 和 Yacoub, Coronary Arterry Disease 8, 389-402, (1997); Hattori 等，J. Mol. Cell. Cardiol. 29, 1585-92(1997)。实际上，据报道，就肌细胞 iNOS 诱导而言，IFN-γ是最有效的细胞因子(Watkins 等，J. Mol. & Cell Cardiol 27, 2015-29(1995))。

心脏肥大

肥大一般指某器官或结构非正常发育的增大，但与肿瘤形成无关。器官或组织肥大或者由单个细胞体积增大引起(真性肥大)，或者由构成组织的细胞数量增多引起(增生)，或者以上两者综合所致。

心脏肥大指机械和激素刺激引起的心脏增大，以使心脏适应增加的心输出或

损伤。Morgan 和 Baker，Circulation 83, 13-52(1991)。这种应答通常伴随着许多不同的病理情况，例如高血压、主动脉狭窄、心肌梗塞、心肌病、瓣膜返流、心脏分流、充血性心力衰竭，等。

在细胞水平上，心脏以合胞体的形式工作，所述合胞体由肌细胞和称为非肌细胞的周围支持细胞构成。肌细胞主要为成纤维细胞/间叶细胞，但也包括内皮细胞和平滑肌细胞。实际上，虽然成人心肌主要由肌细胞构成，但它们只占心脏总细胞数的约 30%。

胎儿心脏的增大主要是因为肌细胞数量的增多，这一过程持续到出生后不久，到心肌细胞失去增殖能力的时候。以后的发展则是单个细胞的增大。成人心室肌细胞肥大是对多种慢性血流动力超负荷的情况的应答。因此，在对激素、生理、血流动力和病理刺激的应答中，成人心室肌细胞能通过激活肥大程序而发生改变以适应增加的工作负荷。这一应答的特征是肌细胞的增大和单个心肌细胞内可收缩蛋白含量的增多，但不伴随细胞分化和胚胎基因(包括心房促尿钠排泄肽(ANP)的基因)的激活。Chien 等，FASEB J. 5, 3037-3046, (1991); Chien 等，Annu. Rev. Physiol. 55, 77-95(1993)。已有记载，在压力过高引起的人左心室肥大中，肌细胞增大引起心肌组织增大，而肌细胞增大则与间质胶原在胞外基质内和心肌内冠状动脉周围的积累有关(Caspari 等，Cardiovasc. Res. 11, 554-8(1977); Schwarz 等，Am. J. Cardiol. 42, 895-903(1978); Hess 等，Circulation 63, 360-371(1981); Pearlman 等，Lab Invest 46，158-164(1982))。大多数心脏病结果都会造成慢性血流动力超负荷所致的心脏肥大，这也是心力衰竭的共同特征。

已有人提出，非肌细胞的支持细胞产生的旁泌因子可能也参与心脏肥大的发展，并且，已经鉴定了许多非肌细胞产生的肥大因子，例如白细胞抑制因子(LIF)和内皮素。Metcalf, Growth Factors 7, 169-173(1992); Kurzrock 等，Endocrine Reviews 12, 208-217(1991); Inoue 等，Proc. Natl. Acad. Sci. USA (1996 年 11 月 12 日出版)。已鉴定的其它因子例如心脏肥大潜在介导物，这包括心脏营养因子-1(cardiotrophin-1，CT-1)(Pennica 等，Proc. Natl. Acad. Sci. USA 92，1142-46(1995))、儿茶酚胺、肾上腺皮质类固醇、血管紧张素和前列腺素。

作为对心脏功能损伤的短期应答，减少单个肌纤维的负荷，成人肌细胞肥大最初是有益的。然而，由于严重的长期超负荷，肥大细胞开始退化和死亡。Katz, "心力衰竭"，Kate A.M.编的 Physiology of Heart(New York, Raven Press, 1992)pp. 638-668。在临床心力衰竭过程中，心脏肥大是突出的致命和致病因子。Katz, Trends Cardiovasc Med. 5, 37-44(1995)。

有关心脏肥大原因和病理的更多细节，参见，例如，Heart Disease, A Textbook of Cardiovascular Medicine, Braunwald, E.编，W.B.Saunders Co., 1988，第 4 章，心力衰竭的病理学。

心脏肥大的治疗

目前，心脏肥大的治疗方法根据所患心脏病而不同。儿茶酚胺、肾上腺皮质类固醇、血管紧张素、前列腺素、白血病抑制因子(LIF)、内皮素(包括内皮素-1、-2、-3 和大内皮素)、心脏营养因子-1(CT-1)和心脏肥大因子(CHF)，这些因子都被认为属于心脏肥大强介导物。例如，β-肾上腺素能受体抑制药(β-抑制剂，例如普奈洛尔、噻吗洛尔、特他洛尔、卡替洛尔、纳多洛尔、倍他洛尔、喷布洛尔、acetoburolol、阿替洛尔、美托洛尔、卡维地洛，等)和维拉帕米已被广泛用于治疗肥厚性心肌病。β-抑制剂对症状(例如胸痛)和运动耐量的改善主要是因为减慢了心率，因而延长了舒张期，增加了心室的被动输入。Thompson 等，Br. Heart J. 44, 488-98(1980); Harrison 等，Circulation 29, 84-89(1964)。维拉帕米据说能增加心室输入和可能减少心肌局部缺血。Bonow 等，Circulation 27,853-64(1985)。有时也用硝苯地平和地尔硫䓬治疗肥厚性心肌病。Lorell 等，Circulation 65，499-507(1982)；Betocchi 等，Am. J. Cardiol. 78, 451-7(1996)。然而，因为具有强血管扩张特性，硝苯地平可能有害，尤其是在输出受阻的患者中。曾利用丙吡胺的负性肌力作用来缓解症状。Pollick, N. Eng. J. Med. 307, 997-9(1982)。然而，在许多患者中，最初的作用会随时间减退。Wigle 等，Circulation 92, 1680-92(1995)。

据报道，用抗高血压药治疗对高血压相关性心脏肥大也有效。例如，单用或联合用于抗高血压疗法的药物有：钙拮抗剂，例如尼群地平；前文所述的β-肾上腺素能受体抑制药；血管紧张素转化酶(AIE)抑制剂，例如喹那普利、卡托普利、依那普利、雷米普利、benazepril、福辛普利、赖诺普利；利尿剂，例如氯噻嗪、氢氯噻嗪、氢氟噻嗪、甲基氯噻嗪、苄噻嗪、双氯非那胺、乙酰唑胺、吲哒帕胺；钙通道抑制剂，例如地尔硫䓬、硝苯地平、维拉帕米、尼卡地平。例如，用地尔硫䓬和卡托普利治疗高血压表现出左室心肌减小，但是舒张期作用的 Doppler 指数未归一。Szlachcic 等，Am. J. Cardiol. 63, 198-201(1989); Shahi 等，Lancet 336, 458-61(1990)。以上发现被解释成是提示：过量间质胶原可能在左心室肥大缓解后留存。Rossi 等，Am. Heart J. 124, 700-709(1992)。Rossi 等(同上)在实验大鼠中研究了卡托普利预防和逆转细胞肥大的作用，对高压力负荷性心脏肥大间质纤维化的作用。

因为没有通用的治疗心脏肥大的方法，鉴定能够预防或减轻心肌细胞肥大的因子在发展新的治疗方法以抑制病理性心脏生长中尤其重要。

分明概述

我们意外发现，IFN-γ抑制大鼠分离细胞受前列腺素 $F_{2\alpha}$($PGF_{2\alpha}$)和苯福林诱导的心肌细胞膨大。我们还发现，IFN-γ在活体大鼠模型体内抑制 $PGF_{2\alpha}$拮抗性类似物氟前列醇诱导的肥大和压力负荷诱导的肥大。

所以，本发明涉及通过给予治疗有效量 IFN-γ治疗各种潜在病因所致的心脏肥大。如果治疗的是人患者，以重组人 IFN-γ(rh IFN-γ)为佳，最好是 rh IFN-γ-1b，见后文。本文中的治疗采用其最广义的概念，具体包括预防、缓解、减轻和治愈各阶段的心脏肥大。

最好给予经保存具有长期稳定性的液体药剂形式的 IFN-γ。保存液体药剂可含多次剂量的 IFN-γ，并因此可以重复使用。

IFN-γ可与一种或多种其它治疗心脏肥大或促进心脏肥大发展的病理状态(例如高血压，大动脉狭窄或心肌梗塞)的药物联用。

本发明还包括制造用于治疗心脏肥大的药物组合物的方法，所述组合物中含IFN-γ作为有效成分。

本发明还涉及一种药物产品，它包括：

(a) 含至少一份治疗有效量 IFN-γ的药物组合物；

(b) 含所述药物组合物的容器；和

(c) 容器所带的标签或所述药物产品内的包装说明书，说明所述 IFN-γ在治疗心脏肥大中的应用。

附图简述

在附图和实施例中，“IFN”或“IFN-γ”指重组鼠 IFN-γ(Genentech, Inc., South San Francisco, CA 或 Genzyme, Cambridge, MA)。

图 1：IFN-γ对前列腺素 $F_{2\alpha}$ ($PGF_{2\alpha}$)诱导的膨大的抑制。分离当日，肌细胞与盐水载体或 IFN-γ(500U/ml)预培养。分离后 24 小时，第二次加载体或 IFN-γ，同时加载体或 $PGF_{2\alpha}$(10^{-7}M)。继续培养 72 小时后，用戊二醛固定细胞，用黄色伊红染色，用荧光显微镜观察。A、B、C 分别是以对照、$PGF_{2\alpha}$和 $PGF_{2\alpha}$+IFN-γ培养 4 天后的心肌细胞。显示杆状心肌细胞最大宽度和呼吸频率百分比的直方图。杆状细胞的最大宽度是用荧光显微镜和图象分析软件确定的。每组至少检查同一实验

的 200 个杆状细胞。IFN-γ单用对细胞形态没有观察得到的影响。所有组间比较的 P<0.001。

图 2：IFN-γ(500-25U/ml)对 $PGF_{2\alpha}$诱导的反应的剂量反应性抑制。分离当日，肌细胞与盐水载体或 IFN-γ预培养。分离后 24 小时，第二次加载体或 IFN-γ，同时加载体或 $PGF_{2\alpha}(10^{-7}M)$。继续培养 72 小时后，用戊二醛固定细胞，用黄色伊红染色，用荧光显微镜观察。细胞形态定量：A 对照，B $PGF_{2\alpha}$，C $PGF_{2\alpha}$+IFN-γ(25U/ml)，D $PGF_{2\alpha}$+IFN-γ(100U/ml)，E $PGF_{2\alpha}$+IFN-γ(500U/ml)。显示杆状心肌细胞最大宽度和呼吸频率百分比的直方图。杆状细胞的最大宽度是用荧光显微镜和图象分析软件确定的。每组至少检查同一实验的 200 个杆状细胞。IFN-γ单用对细胞形态没有观察得到的影响。所有组间比较的 P<0.001。

图 3：IFN-γ对苯福林(PE)诱导的膨胀的抑制。分离当日，肌细胞与盐水载体或 IFN-γ(500U/ml)预培养。分离后 24 小时，第二次加载体或 IFN-γ，同时加载体或 $PE(10^{-5}M)$。继续培养 72 小时后，用戊二醛固定细胞，用黄色伊红染色，用荧光显微镜观察。A、B、C 分别是以对照、PE 和 PE+IFN-γ培养 4 天后的心肌细胞。显示杆状心肌细胞最大宽度和呼吸频率百分比的直方图。杆状细胞的最大宽度是用荧光显微镜和图象分析软件确定的。每组至少检查同一实验的 200 个杆状细胞。IFN-γ单用对细胞形态没有观察得到的影响。所有组间比较 P<0.001。

图 4：大鼠体内，IFN-γ对氟前列醇诱导的心脏肥大的作用。所示数据是平均值±SEM。括弧内是每组的动物数。与载体组相比，*P<0.05，*P<0.01。与 Flup 组相比，#P<0.05，##P<0.01。Flup：氟前列醇；IFN= IFN-γ；HW：心脏重量；BW：体重；VW：心室重量；LVW 左心室重量。

图 5：Flup 和/或 IFN 对 MAP 和 HR 的作用。所示数据是平均值±SEM。括弧内是每组的动物数。与载体组相比，*P<0.05。与 Flup 组相比，#P<0.05。与 Flup+IFN 组相比，+P<0.05。Flup：氟前列醇；IFN= IFN-γ；MAP：平均动脉压；HR：心率。

图 6：显示氟前列醇(FLUP)和 IFN-γ对以下物质表达的作用的直方图：A 骨骼肌动蛋白(SKA)；B 肌质网钙 ATPase(SRCA)；C 胶原 I(COLI)；D 心房利钠因子(ANF)。将表达水平相对戊二醛-3-磷酸酯脱氢酶(GAPDH)信使归一。VEH 是载体。每组有 7 只动物，数据表示为平均值±SEM。对 VEH 组的 P<0.05。

图 7：高压力负荷大鼠体内，IFN-γ对心脏重量、心室重量和左心室重量的作用。数据表示为平均值±SEM。括弧内是每组的动物数。与假处理组相比，**P<0.01。与结扎+载体组相比，## P<0.01。结扎组：大动脉被结扎的大鼠；IFN：

IFN-γ；WH：心脏重量；VW：心室重量；LVW 左心室重量。

图 8：高压力负荷大鼠体内，IFN-γ对心脏重量、心室重量和左心室重量与体重之比的作用。数据表示为平均值±SEM。括弧内是每组的动物数。与假处理组相比，**P<0.01。与结扎+载体组相比，## P<0.01。结扎组：大动脉被结扎的大鼠；IFN：IFN-γ；WH：心脏重量；VW：心室重量；LVW 左心室重量。

图 9：压力负荷大鼠体内，IFN-γ对动脉收缩压、平均动脉压和动脉舒张压的作用。括弧内是每组的动物数。与假处理组相比，**P<0.01。结扎组：大动脉被结扎的大鼠；IFN：IFN-γ；MAP：平均动脉压；DAP：动脉舒张压。

本发明的详细描述

A. 定义

“γ干扰素”、“干扰素γ”或“IFN-γ”指在任意心脏肥大试验(例如本文所述的肥大试验)中表现出生物活性的γ干扰素(人的和其它动物的)的所有形式，这包括但不限于天然、化学合成或重组 DNA 技术产生的成熟、前、变和/或去(1-3)(又称去 CysTyrCys IFN-γ)形式。对制备重组人 IFN-γ(rhu IFN-γ的全面描述(包括其 cDNA 和氨基酸序列)参见，例如，美国专利 4,727,138，4,762,791，4,925,554，5,582,824，5,096,705，4,855,238，5,574,137 和 5,595,888。有关无 CysTyrCys 重组人 IFN-γ，包括差异截断衍生物，参见欧洲专利公布 146,354。非人动物干扰素，包括 IFN-γ，参见欧洲专利公布 88,622。这包括天然(野生型)干扰素的差异糖基化形式和其它变异体(例如氨基酸序列变异体)及衍生物，这些或者已知，或者将会得到。所述变异体例如等位基因，产生残基缺失、插入和/或被取代的定点诱变产物(参见，例如欧洲专利公布 146,354)。已知 IFN-γ的宿主范围很窄，所以，必须使用与被处理动物同源的 IFN-γ。治疗人时，最好使用美国专利 4,717,138 及其同族 EP77,670 所示序列的去 CysTyrCys 变异体，而且或选的是翻译后最后四个残基缺失的 C 末端变异体。对人的治疗而言，本发明的 IFN-γ最好是重组人 IFN-γ(rhu IFN-γ)，有或没有 N 末端的氨基酸 CysTyrCys。更好的是，IFN-γ是作为市售制剂 Actimmune®(Genentech, Inc., South San Francisco, Califonia)中有效成分的重组人 IFN-γ(重组人干扰素γ-1b、rhu IFN-γ-1b，含 140 个氨基酸)。因为已知 IFN-γ具有高度的种属特异性，所以在动物实验或对兽用来说，最好使用被处理动物的同种 IFN-γ。因此，在采用大鼠动物模型的体内实验中，使用的是鼠(小鼠)重组 IFN-γ(Genentech, Inc.)。大鼠与小鼠的相关性足够密切，可以将小鼠 IFN-γ用于大鼠模型。

从药理学上说，在本发明中，“治疗有效量”的 IFN-γ指在治疗肥大，尤其是心脏肥大中有效果的量。

“肥大”在此指不依赖于自然发育且与肿瘤形成无关的器官或结构的增大。器官或组织的肥大可能是因为单个细胞的增大(真性肥大)，也可能是因为构成该组织或器官的细胞数量的增多(增生)，或两者综合所致。某些器官，例如心脏，出生后不久就失去了分裂的能力。所以，“心脏肥大”是心脏体积的增大，在成人中，表现为肌细胞增大和收缩蛋白含量增多，但不伴随细胞分裂。可诱发肥大的应激(例如前负荷增加，后负荷增加，象心肌梗塞中那样的肌细胞减少，或收缩抑制)的特征似乎对反应的特征具有重要作用。心脏肥大的早期通常表现为形态上肌原纤维和线粒体的增大，以及线粒体和细胞核的增大。此时，虽然肌细胞比正常的大，细胞组织基本保留。在心脏肥大的进一步阶段，特定细胞器，例如线粒体增大或增多，而且，新的收缩元素以无规律的方式增加到局部区域。长期肥大的细胞表现出更明显的细胞组织异常，包括明显的核增大和膜的高度分叶，这取代了附近的肌原纤维，造成正常 Z 带记录的破坏。“心脏肥大”包括以心肌不同程度结构损伤为特征的病情发展的各阶段，与所患心脏病无关。

“心力衰竭”指一种心脏功能异常，即心脏泵送血液的速度达不到组织代谢的要求。心力衰竭可能由许多原因造成，包括缺血、充血、风湿或原发性的。

“充血性心力衰竭”是一种进行性病理状态，心脏越来越无法提供足够的心输出(即单位时间内心脏泵送的血液量)为周围组织提供含氧血。随着充血性心力衰竭的发展，出现结构和血流动力学损伤。虽然这些损伤的表现形式不同，特征性症状之一是心室肥大。许多不同的心脏病最后都会造成充血性心力衰竭。

“心脏肥大”的原因通常是冠状动脉粥样硬化，常伴有冠状动脉血栓形成。可将其分为两个重要类型：透壁梗死，即心肌坏死涉及心室的整个厚度；心内膜下梗死(非透壁梗死)，即坏死只涉及心内膜下膜或心室内心肌或以上两者，但没有穿过整个心室壁达到心外膜。已知，心肌梗塞既引起血流动力学改变又造成心脏受损和健康区域的结构改变。因此，心肌梗塞减少最大心输出和心脏射血量。与心肌梗塞相关的还有发生在间质内的 DNA 合成刺激，以及心脏未受影响部位胶原形成增加。

在例如总外周阻力增加引起的长期高血压中，作为应激或紧张度增加的结果，一直将心脏肥大与高血压相关联。长期压力过高所致心室肥大的特征之一是舒张功能不全。Fouad 等，J. Am. Coll. Cardiol. 4, 1500-6(1984)；Smith 等，J. Am. Coll. Cardiol. 5, 869-74(1985)。在早期原发性高血压中已经检测到了左心室舒张延

长，但收缩功能正常或超常。Hartford 等，Hypertension 6, 329-338(1984)。然而，血压水平和心脏肥大之间缺乏密切的平行关系。虽然，有报道称，对人的抗高血压治疗可改善左心室功能，但已发现，分别用利尿剂(氢氯噻嗪)、β抑制剂(普萘洛尔)或钙通道抑制剂(地尔硫䓬)治疗的患者左心室缩小，舒张功能却无改善。Inouye 等，Am. J. Cardiol. 53, 1583-7(1984)。

与心脏肥大相关的另一种复杂的心脏病是“肥厚性心肌病”。该病的特征是形态、功能和临床症状具有极大的多样性(Maron 等， 780-9(1987)；Spirito 等，N. Eng. J. Med. 320, 749-55(1989)；Louie 和 Edwards, Prog. Cardiovasc. Dis. 36, 275-308(1994)；Wigle 等，Circulation 92, 1680-92(1995))，它可发生于各种年龄的患者，这更增加了它的多样性(Sprito 等，N. Eng. J. Med. 336, 775-785(1997))。肥厚性心肌病的病因也很多，而且知道的很少。最近的信息表明，β肌球蛋白重链突变可能是30-40%家族性肥厚性心肌病的病因(Watkins等，N. Eng. J. Med. 326, 1108-14(1992)；Schwartz 等，Circulation 91, 532-40(1995)；Marian 和 Roberts, Circulation 92, 1336-47(1995)；Thierfelder 等，Cell 77, 701-12(1994)；Watkins 等，Nat. Gen. 11, 434-7(1995))。

瓣膜上方的“主动脉狭窄”是一种遗传性血管病，特征为上行主动脉狭窄，但其它动脉，包括肺动脉，也可能受到影响。主动脉狭窄如果不治疗会造成心内压力升高，导致心肌肥大，最后造成心力衰竭和死亡。对该病的病因还未完全掌握，但内层平滑肌肥大和可能的增生是该病的突出特征。据报道，弹性蛋白的变异分子与主动脉狭窄的发展和病因有关。(1997 年 7 月 22 日的美国专利 5,650,282)。

“瓣膜返流”是造成心瓣膜异常的心脏病所致。多种疾病，例如风湿性高烧，会造成瓣膜口收缩或拉开，其它则可能造成心内膜炎，即心内膜或房室口内膜发炎，并需要心脏手术。瓣膜狭窄之类缺陷或瓣膜闭合不全造成血液在心腔内积聚或者通过瓣膜返流。如果不加以矫正，长期的瓣膜狭窄或功能不全会造成心脏肥大相关的心肌损伤，最后可能需要替换瓣膜。

本发明的主题就是处理所有这些及其它伴有心脏肥大的心脏病。

“治疗”包括以避免或减缓(缓解)肥大为目的的治疗和预防。需要治疗的包括已经患病的，易于患病的，或者需要避免该病的。肥大可以是多种原因的，包括自发的、心脏病的、缺血性的或缺血性发作，例如心肌梗塞。

“慢性”给药指与急性方式相对的连续给予药物，从而在更长的时间内保持最初的抗肥大作用。

“哺乳动物”，就治疗目的而言，指归为哺乳动物的任意动物，包括人、圈养或放养的动物、以及观赏、运动或宠物类动物，例如狗、猫、牛、马、羊、猪，等。最好是人。

与一种或多种其它药物“联用”包括同时给药和任意次序的先后给药。

B. 最佳实施方式

1. 心脏肥大试验

体外试验

a. 成年大鼠心肌细胞膨大诱导

该试验中，分离出一个大鼠(雄性，Sprague-Dawley)的心室肌细胞，基本上按照 Piper 等“成年大鼠心室肌细胞”Cell Culture Techniques in Heart and Vessel Research, H.M.Piper 编，Berlin: Springer-Verlag, 1990, pp.36-60 所述方法的修改形式。给方法能够分离出成年心室肌细胞并长期培养杆状表型的这些细胞。已知苯福林和前列腺素 $F_{2\alpha}$($PGF_{2\alpha}$)能在这些细胞中诱导膨大反应。Piper 等，同上；Lai 等，Am. J. Physiol. 1996；217(Heart Circ，Physiol.40): H2197-2208。然后测定心脏肥大的不同潜在抑制剂对 $PGF_{2\alpha}$或 $PGF_{2\alpha}$类似物(例如氟前列醇)和苯福林诱导的肌细胞膨大的抑制。

体内试验

a. 体内对氟前列醇诱导心脏肥大的抑制

该药学模型测试 IFN-γ在大鼠(例如雄型，Wistar 或 Sprague-Dawley)体内抑制皮下注射氟前列醇($PGF_{2\alpha}$类似物之一)诱导的心脏肥大的能力。已知，患心肌梗塞诱导的病理性心脏肥大的大鼠其心肌内已逐渐积聚了高水平的可抽提 $PGF_{2\alpha}$。Lai 等，Am. J. Physiol.(Heart Circ. Physiol.)271，H2197-H2208(1996)。所以，能够在体内抑制氟前列醇对心肌生长作用的因素可能可用于治疗心脏肥大。IFN-γ对心脏肥大的作用通过测定心脏、心室和左心室(相对体重归一)的重量并与不接受 IFN-γ的大鼠进行比较来确定。实施例中提供了该试验的详细描述。

b. 压力-超负荷心脏肥大试验

对体内测试而言，一般通过测试动物腹主动脉收缩来诱导压力-超负荷心脏肥大。在一典型方案中，大鼠(雄型，Wistar 或 Sprague-Dawley)接受麻醉，将横膈下的腹主动脉缝窄。Benznak M.，Can. J. Biochem. Physiol. 33, 985-94(1955)。手术切开主动脉，将钝针放在血管旁边。用羊毛结扎线缠绕钝针，然后立即去除钝针，于是主动脉腔被收窄为针的直径。该方法参见 Rossi 等，Am. Heart J. 124,

700-709(1992)和 O'Rourke 和 Reibel, P.S.E.M.B., 200, 95-100(1992)。本发明所用方法的详细描述参见后文实施例。

b．实验诱导心肌梗塞(MI)对心脏肥大的影响

在大鼠体内通过左冠状动脉结扎诱导急性 MI，并用心电图检查进行确认。同时准备一假处理组动物作为对照。早期数据显示心脏肥大存在于 MI 动物组，表现为心脏重量与体重之比升高 18%。Lai 等，同上。用心脏肥大候选抑制剂，例如 IFN-γ，处理这些动物，提供了有价值的关于测试候选抑制剂疗效的信息。

2．IFN-γ的用途、治疗组合物和给药

根据本发明，IFN-γ可用来治疗心脏肥大，即各种病因和病理的心脏增大。当心脏(心室)承受过高的压力或容量负荷时，心脏(心肌)肥大就会发生，作为一种基本补偿机制，使得心室能够承受它的负荷。Krayenbuehl 等，Eur. Heart J. 4(增刊 A)，29(1983)。诱发肥大的应激(例如前负荷增加，后负荷增加，象心肌梗塞中那样的肌细胞减少，或收缩性主抑制)的特征对肥大反应的特征具有重要作用。Scheuer 和 Buttrick, Circulation 75(增刊 1)，63(1987)。本发明涉及与任意病理状况相关的心脏肥大的治疗，所述状况包括但不限于心肌梗塞后，高血压，主动脉狭窄，心肌病，瓣膜返流，心脏分流术和充血性心力衰竭。前文已论述过这些状况的主要特征。

特别重要的是在心肌梗塞后用 IFN-γ预防心力衰竭。在美国，每年约 750,000 人患急性心肌梗塞(AMI)，约四分之一因 AMI 而死亡。最近几年，溶血栓剂，例如链激酶、尿激酶，尤其是组织血纤蛋白溶酶原(t-PA)大大提高了心肌梗塞患者的存活率。连续静脉输注 1.5 至 4 小时，在 69-90%受治患者中，t-PA 在第 90 分钟造成冠状动脉开通。Topol 等，Am. J. Cardiol. 61, 723-8(1988)；Neuhaus 等，J. Am. Coll. Cardiol. 12, 581-7(1988)；Neuhaus 等，J. Am. Coll. Cardiol. 14, 1566-9(1989)。据报道，采用高剂量或加快给药可达到最快开通速度(rate)。Topol 等，Am. J. Cardiol. 15, 922-4(1990)。t-PA 也可以作为药丸给予，但因为其半衰期较短，所以更适合采用输注疗法。Tebbe 等，Am. J. Cardiol. 64, 448-53(1989)。特别适合药丸给药的一种具有更长的半衰期和很高的血纤维蛋白特异性 t-PA 变异体，TNK t-PA(T103N，N117Q，KHRR(296-299)AAAA t-PA 变异体，Keyt 等，Proc. Natl. Acad. Sci. USA. 91, 3670-3674(1994))。然而，尽管已取得以上进展，存活患者的长期预后主要取决于梗塞后的监护和对患者的治疗，这应当包括对心脏肥大的监测和治疗。

另一重要的治疗内容是治疗与高血压相关的心脏肥大。如前所述，已知长期

的高血压会导致心脏肥大。虽然已知有些高血压药能够减小左心室，但治疗并不一定改善舒张功能。所以，IFN-γ可联合β肾上腺素能受体抑制剂，例如普奈洛尔、噻吗洛尔、特他洛尔、卡替洛尔、纳多洛尔、倍他洛尔、喷布洛尔、acetoburolol、阿替洛尔、美托洛尔、卡维地洛；血管紧张素转化酶(AIE)抑制剂，例如喹那普利、卡托普利、依那普利、雷米普利、benazepril、福辛普利、赖诺普利；利尿剂，例如氯噻嗪、氢氯噻嗪、氢氟噻嗪、甲基氯噻嗪、苄噻嗪、双氯非那胺、乙酰唑胺、吲哒帕胺；钙通道抑制剂，例如地尔硫䓬、硝苯地平、维拉帕米、尼卡地平。包含以上通用名所示疗效药的药物组合物可以购买得到，并可按照生产商有关剂量、剂型、副作用、禁用症等的说明给药(参见，Physician's Desk Reference, Medical Economics Data Production Co. Montvale N.J., 第 51 版，1997)。

IFN-γ可以预防性地给予心脏肥大患者，用于阻止病情的发展和避免猝死，包括无症状性猝死。这样的预防性治疗对于诊断为巨大性左心室肥大(成人的最大壁厚达 35mm 或以上，或者儿童的相当壁厚)的患者，或者心脏的血流动力学负担特别重时，尤其必要。

IFN-γ还可以用于治疗大多数确诊为肥厚性心肌病的患者都会发生的心房纤颤。

IFN-γ以药物组合物的形式给予，其中包含 IFN-γ作为有效成分，还包括药学上认可的载体。用于治疗心脏肥大的治疗性 IFN-γ制剂如下制备以便保存：将纯度达到要求的 IFN-γ与或选性的药学上认可的载体、赋形剂或稳定剂(Remington's Pharmaceutical Science, 同上)混合，制备成冻干块或水溶液形式。认可的载体、赋形剂和稳定剂在使用剂量和浓度对受主无毒，包括缓冲液，例如磷酸、柠檬酸和其它有机酸；抗氧化剂，包括抗坏血酸；低分子量(少于 10 个残基)的肽；蛋白质，例如血清白蛋白、明胶或免疫球蛋白；亲水性聚合物，例如聚乙烯吡咯烷酮；氨基酸，例如甘氨酸、谷氨酰胺、门冬酰胺、精氨酸或赖氨酸；单糖，二糖和其它糖，包括葡萄糖、甘露糖或糊精；螯合剂，例如 EDTA；糖醇，例如甘露醇或山梨醇；自动生成反离子，例如钠；和/或非离子表面活性剂，例如 Tween，Pluronic 或聚乙二醇(PEG)。

体内给药的 IFN-γ必须是无菌的。在冷冻干燥和再生之前或之后经灭菌滤膜过滤即可做到这一点。一般以冻干或溶液形式保存 IFN-γ。

可使用冻干形式的 IFN-γ，并与其它成分混合以便用合适的溶剂在使用时再生。因为已知 IFN-γ对酸不稳定，一般在中性或弱碱性 pH 下进行处理。参见，例如，美国专利 4,499,014，其中描述了冻干酸性 IFN-γ溶液到达 pH6-9 时的重新活

化。中性或弱碱性的高浓度 IFN-γ溶液一般不适于作为输注制剂，因为会迅速形成可见沉淀。这种沉淀会在给药时造成栓塞或降低药效。欧洲专利公布 0196,203 公开了冻干 IFN-γ在 pH4-6.0 的再生。

1992 年 9 月 29 日的美国专利 5,151,265 公开的稳定液体药物组合物含有效量的非冻干 IFN-γ，能维持 pH4.0-6.0 的缓冲液，稳定剂和非离子去污剂。稳定剂一般是多元糖醇，例如甘露醇，非离子去污剂可以是表面活性剂，例如聚山梨醇酯 80 或聚山梨醇酯 20。非离子去污剂的含量以约 0.07-0.2mg/ml 为宜，最好是 0.1mg/ml。合适的缓冲液是有机酸及其盐组成的常规缓冲液，例如柠檬酸盐缓冲液(例如，柠檬酸一钠-柠檬酸二钠混合物，柠檬酸-柠檬酸三钠混合物，柠檬酸-柠檬酸一钠混合物，等)，琥珀酸盐缓冲液(例如，琥珀酸-琥珀酸一钠混合物，琥珀酸-氢氧化钠混合物，琥珀酸-琥珀酸二钠混合物，等)，酒石酸盐缓冲液(例如，酒石酸-酒石酸钠混合物，酒石酸-酒石酸钾混合物，酒石酸-氢氧化钠混合物，等)，延胡索酸盐缓冲液(例如，延胡索酸-延胡索酸一钠混合物，延胡索酸-延胡索酸二钠混合物，延胡索酸一钠-延胡索酸二钠混合物，等)，葡糖酸盐缓冲液(例如，葡糖酸-葡糖酸钠混合物，葡糖酸-氢氧化钠混合物，葡糖酸-葡糖酸钾混合物，等)，草酸盐缓冲液(例如，草酸-草酸钠混合物，草酸-氢氧化钠混合物，草酸-草酸钾混合物，等)，乳酸盐缓冲液(例如，乳酸-乳酸钠混合物，乳酸-氢氧化钠混合物，乳酸-乳酸钾混合物，等)，和乙酸盐混合物(例如，乙酸-乙酸钠混合物，乙酸-氢氧化钠混合物，等)。

一种已知的市售 IFN-γ液体制剂(Actimmune® rhu IFN-γ-1b, Genentech, Inc.)是一种无菌、澄清、无色、非储备溶液，装在单剂量小瓶中用于皮下注射。每 0.5ml Actimmune®含 100μg(3×10^6 单位，比活性：30×10^6 单位/mg) IFN-γ-1b，以 20mg 甘露醇、0.36mg 琥珀酸钠、0.05mg 聚山梨醇酯 20 和无菌注射水配制。

适合重复使用的，按照本发明使用的储备药物组合物，宜包含：

a)非冷冻干燥的 IFN-γ；

b)能维持 pH 约 4-6(即蛋白质在溶液中稳定性最高的 pH 范围)的乙酸盐缓冲液；

c)非离子去污剂，主要用于稳定蛋白质，抵抗搅拌造成的凝聚；

d)等渗调节剂；

e)防腐剂，选自：苯酚、苯甲醇和卤化苄乙氧铵，例如氯化苄乙氧铵；和

f)水。

非离子去污剂(表面活性剂)可以是例如聚山梨醇酯(例如聚山梨醇酯

(Tween)20 或 80)或泊洛沙姆(例如泊洛沙姆 188)。用非离子表面活性剂能使制剂不致因表面剪切力而造成蛋白质变性。此外，含此类表面活性剂的制剂可用于气雾装置，例如肺给药时所用的，和无针注射枪(参见，EP257,956)。

含等渗调节剂是为了确保本发明液体组合物的等渗性，它们包括多元糖醇，例如三元或更多元的糖醇，例如甘油、赤藓醇、阿拉伯醇、木糖醇、山梨醇和甘露醇。这些糖醇可以单用也可联用。或者，可用氯化钠或其它合适的无机盐来维持溶液的等渗性。

乙酸缓冲液可以是，例如，乙酸-乙酸钠混合物，乙酸-氢氧化钠混合物，等等。本发明液体制剂的 pH 在 4.0-6.0 之间，pH4.5-5.5 更好，pH5 则最好。

防腐剂，例如苯酚、苯甲醇和卤化苄乙氧铵，例如氯化苄乙氧铵是已知的抗微生物剂。

在一优选实施例中，给予的 IFN-γ是液体药物组合物的形式，其中含：

IFN-γ	0.1-2.0mg/ml
乙酸钠(pH5.0)	5-100mM
Tween 20	0.1-0.01wt%
苯酚	0.05-0.4wt%
甘露醇	5wt%
注射水，USP	补足至 100%

其中，百分比含量的基础是组合物的总重。苯酚可用 0.5-1.0wt%苯甲醇代替，甘露醇可用 0.9wt%氯化钠代替。

最好，组合物含：

IFN-γ	0.1-1.0mg/ml
乙酸钠(pH5.0)	10mM
Tween 20	0.01wt%
苯酚	0.2wt%
甘露醇	5wt%

苯酚可用 0.75wt%苯甲醇代替，甘露醇可用 0.9wt%氯化钠代替。

储备液制剂最好含多剂次治疗有效量的 IFN-γ。因为该多肽的宿主范围很窄，治疗人患者，宜使用含人 IFN-γ的液体制剂，含天然序列人 IFN-γ的更好。作为生物反应修饰剂，IFN-γ对人和非人哺乳动物的多种类型细胞具有多种活性。当然，治疗有效量取决于多种因素，例如受治(包括预防)病理状况、患者年龄、体重、总体健康状况、医疗史，等，开业医师能够对此做出决定。有效剂量一般约

0.001-1.0mg/kg，约 0.01-1mg/kg 更好，约 0.01-0.1mg/kg 最好。以 A549 细胞进行抗脑心肌炎病毒试验时，在这样的制剂中，hu IFN-γ较好地表现出高于或等于约 2×10^7U/mg 蛋白质的比活性。应该知道的是，必须将内毒素污染维持在安全水平以下，例如低于 0.5ng/mg 蛋白。而且，若是给予人，液体制剂必须符合 FDA 和生物学标准提出的无菌、热原、总体安全性和纯度要求。

IFN-γ的给药途径采用已知方法，例如静脉、腹膜内、脑内、肌内、眼内、动脉内或创伤内注射或输液，或采用后文所述的缓释系统。一般将疗效性 IFN-γ组合物装在具有无菌接口的容器内，例如静脉输液袋或塞子可被皮下注射针穿通的管形瓶。制剂的给予最好是多次静脉(i.v.)、皮下(s.c.)或肌内(i.m.)注射，或是适合鼻内或肺内(有关肺内给药，参见 EP257,956)给药的气雾剂。

稳定的 IFN-γ水性组合物最好装在管形瓶中，含多至约 30 剂治疗有效量的 IFN-γ。首此给药后至少 14 天内，至少 200 天更好，IFN-γ的生物活性最好能保持首此给药使所表现活性的约 80%。

IFN-γ还可以缓释剂的形式给予。缓释剂的合适离子包括含蛋白质的固体疏水聚合物半透过性基质，该机制具有一定形状，例如膜或微胶囊。缓释基质的例子包括聚酯，水凝胶(例如聚(2-羟乙基-甲基丙烯酸酯))(Langer 等，J. Biomed. Mater. Res., 15, 167-277(1981)和 Langer, Chem. Tech., 12, 98-105(1982))，或聚(乙烯醇)，聚交酯(美国专利 3,773,919，EP58,481)，L-谷氨酸和 L-谷氨酸γ乙酯的共聚物(Sidman 等，Biopolymers 22: 547-556(1983))，不可降解乙烯乙酸乙酯(Langer 等，同上)，可降解乳酸-乙醇酸共聚物，例如 Lupron Depot™(乳酸-乙醇酸共聚物和 leuprolide 乙酸酯构成的可注射微球)，聚-D-(-)-3-羟基丁酸(EP133,988)。

聚合物，例如乙烯乙酸乙酯和乳酸-乙醇酸能长达 100 天地释放分子，有些水凝胶则能持续较短时间地释放蛋白质。当被包裹蛋白在体内滞留较长时间后，它们会因为处于 37℃的潮湿环境中而变性或凝聚，造成生物活性损失，并可能改变免疫原性。可根据有关机制，设计合理的给药方案以确保蛋白质的稳定。例如，如果发现凝聚机制是通过硫-二硫化物互变形成了二硫键，稳定化可通过修饰巯基残基、冷冻干燥酸性溶液、控制湿度、采用合适的添加剂和发明特殊聚合物基质组合物。

缓释 IFN-γ组合物还包括脂质体包裹的 IFN-γ。含 IFN-γ的脂质体可用已知方法制备：DE3,218,121；Epstein 等，Proc. Natl. Acad. Sci. USA, 82: 3688-3692(1985)；Hwang 等，Proc. Natl. Acad. Sci. USA, 77: 4030-4034(1980)；EP52,322；EP36,676；EP88,046；EP143,949；EP142,641；日本专利公告 83-118008；美国专利 4,485,045

和 4,544,545；和 EP102,324。一般采用小型(约 200-800 埃)单片层型的脂质体，其中的脂质含量是 30mol%以上的胆固醇，所选比例根据优化疗法来调节。

用于治疗的有效量 IFN-γ取决于，例如，治疗目的、给药途径和患者状况。所以，为了获得最佳的治疗效果，医生有必要确定剂量和修改给药途径。给予 IFN-γ治疗慢性肉芽肿患者时，体表面积 $0.5m^2$ 以上患者的推荐给予剂量是 $50mcg/m^2(1.5\times10^6U/m^2)$，体表面积小于或等于 $0.5m^2$ 患者的推荐给予剂量是 1.5mcg/kg/剂，皮下注射，每周 3 次。这对于医师确定心脏肥大最佳有效剂量来说具有指导意义。医生将给予 IFN-γ，直到获得治疗心脏功能失调效果所需的剂量。例如，如果目的是治疗充血性心力衰竭，就应是能抑制与这种状况相关的进行性心脏肥大的量。以上疗法的进展过程可方便地通过超声心动图来监测。同理，在肥厚性心肌病患者中，可凭经验，根据患者对疗效的主观感受来给予 IFN-γ。

IFN-γ可联合其它用于治疗(包括治疗)心脏肥大的疗效药一起给药。例如，IFN-γ治疗可与已知的心脏肌细胞肥大因子抑制剂(例如α-肾上腺素能活化剂的抑制剂，例如苯福林；内皮肽-1；CT-1；LIF；血管舒张肽转化酶和血管舒张肽 II)联合给药。对联合疗法来说，特别好的是联合心脏肥大因子抑制剂(CHF，cardiotrophin 或 cardiotrophin-1，参见美国专利 5,679,545)。

心脏肥大联合疗法中的优选候选药物是β肾上腺素能受体抑制剂(例如普奈洛尔、噻吗洛尔、特他洛尔、卡替洛尔、纳多洛尔、倍他洛尔、喷布洛尔、acetoburolol、阿替洛尔、美托洛尔、卡维地洛)，维拉帕米，difedipine，地尔硫䓬。治疗与高血压相关的心脏肥大可能需要采用抗高血压疗法，采用钙通道抑制剂，例如地尔硫䓬、硝苯地平、维拉帕米、尼卡地平；β肾上腺素能阻断剂；利尿剂，例如氯噻嗪、氢氯噻嗪、氢氟噻嗪、甲基氯噻嗪、苄噻嗪、双氯非那胺、乙酰唑胺、吲哒帕胺；和/或 ACE 抑制剂，例如喹那普利、卡托普利、依那普利、雷米普利、benazepril、福辛普利、赖诺普利。

与 IFN-γ联合给予的疗效药物的有效量由医生或兽医确定。为了获得最佳效果，可进行剂量的控制与调整，最好考虑到利尿剂或洋地黄的使用以及高血压、低血压、肾功能不全等因素。剂量还取决于诸如所用疗效药的类型和受治患者的具体状况等因素。通常，所用剂量与不与 IFN-γ联用时的剂量相同。

实施例

实施例 1

IFN-γ对 $PGF_{2\alpha}$诱导的成年肌细胞膨大的抑制

材料与方法

成年肌细胞培养物

分离成年大鼠的心室肌细胞使用的是改进型 Piper 等(同上)所述的方法，详细描述可参见 Lai 等(同上)。为制备每一份肌细胞制剂，用戊巴比妥钠麻醉体重约 250g 的雄性 Sprague-Dawley 大鼠，取出心脏。剔除外围组织，将它固定到温度控制在 37℃的 Langendorff 系统上。用 40ml Krebs 缓冲液(110mM NaCl，2.6mM KCl，1.2mM $MgSO_4 \cdot 7H_2O$，25mM $NaHCO_3$和 11mM 葡萄糖)灌注心脏。然后用含 30mg 胶原酶和 12.5μl 100mM $CaCl_2$的 Krebs 缓冲液在心脏内循环 30 分钟。从 Langendorff 仪上取下心脏，去除心房和结缔组织。用解剖剪将心室切割成 2mm 的小块，然后用新鲜的胶原酶溶液(30mg 胶原酶和 400mg BSA 溶于含 12.5μl 100mM $CaCl_2$的 Krebs 缓冲液)在 37℃消化 5 分钟。消化期间，每分钟温和地手工振荡组织悬浮液。消化后，分离并保留上清液，留下的组织以新鲜胶原酶溶液再消化 5 分钟。

将分离出的成年大鼠肌细胞铺在涂有海带氨酸的平板上，密度为 3×10^3细胞/ml。适当刺激 72 小时后，用甘油醛固定细胞，用黄色曙红染色。在荧光显微镜下查找杆状细胞，以图象分析软件(Simple 32, Compix Imaging, Mars, PA)测定最大宽度。

结果

IFN-γ抑制肥大因子 $PGF_{2\alpha}$和苯福林诱导的成年心肌细胞膨大

已知，$PGF_{2\alpha}$和α肾上腺素能活化剂苯福林诱导培养的成年大鼠新生肌细胞的膨大(Adams 等，J. Biol. Chem. 271: 1179-1186(1996); Lai 等，Am. J. Physiol.(Heart Circ. Physiol.) 271: H2197-2208(1996)；Meidell 等，Am. J. Physiol. 251：H1076-H1084(1986)；Simpson, J. Clin. Invest. 72：732-738(1983)；Simpson, Circ. Res. 56: 884-894(1985))。培养中的成年大鼠心室肌细胞与以上因素接触时膨大(Lai 等，同上；Piper 等，“成年大鼠心室肌细胞”，Cell Culture Techniques in Heart and Vessel Research, H.M.Piper 编，1990，Springer-Verlag: Berlin, p.36-60)。成年肌细胞呈杆状。当这些细胞处于 0.1μM $PGF_{2\alpha}$中时，杆状细胞展平膨大(图 1)。量化膨大反应：测定至少 200 个杆状细胞的最大细胞宽度，将该值相对其在细胞群中的出现频率做图。$PGF_{2\alpha}$明显改变最大细胞宽度，表现为，该值在细胞群内的分布与对照细胞相比发生漂移($p<0.001$)。用 IFN-γ处理细胞明显抑制它们对 $PGF_{2\alpha}$的应答($PGF_{2\alpha}$+ =IFN-γ与 $PGF_{2\alpha}$相比，$p<0.001$)。在一定浓度范围内，IFN-γ对 $PGF_{2\alpha}$诱导

的肌细胞膨大的抑制作用具有剂量依赖性，这符合心肌细胞和其它细胞系统内对IFN-γ的生物反应(Singh 等，Biol. Chem. 271:1111-1117(1996)；Pinsky 等，J. Clin. Invest. 95:766-685(1995)；Ungureanu-Longrois 等，Circ. Res. 77:494-502(1995)；Soderberg-Naucler 等，J. Clin. Invest. 100:3154-3163(1997)；Gou 等，J. Clin. Invest. 100: 829-838(1997)；Marra 等，Can.J. Cardiol. 12: 1259-1267(1996))。抑制 $PGF_{2\alpha}$ 诱导的肌细胞膨大的能力似乎为 IFN-γ所特有，因为包括 IL-1α、IL-1β、IL-2、IL-6、TNF-α、INF-α和 IFN-β在内的其它细胞因子都不抑制膨大反应。IFN-γ的抑制效果并不仅限于对 $PGF_{2\alpha}$。IFN-γ也抑制苯福林诱导的膨大(图 3)。

实施例 2

体内抑制心脏肥大

材料与方法

动物

整个试验过程都符合美国生理学协会的规定，并得到 Genentech 研究动物看护和使用委员会的认可。试验所用的是 Sprague/Dawley(SD)大鼠(8 周龄，Charles River Breeding Laboratories, Inc.)。实验前，动物在试验室内适应至少 1 周，饲以丸状鼠食，自由饮水，笼养于控光控温的房间内。

给予氟前列醇和/或 IFN-γ

给大鼠皮下注射 0.15mg/kg 氟前列醇(Cayman Chemical, Ann Arbor, M)，0.008mg/kg 鼠 IFN-γ(Genentech Inc., South San Francisco, CA)，氟前列醇和鼠 IFN-γ、或盐水载体，每日 2 次，共 14 天。在 IFN-γ组和氟前列醇+IFN-γ组中，动物预先进行 1 天的 IFN-γ预处理。测定处理前后的体重。以前的研究显示，此处所用的氟前列醇剂量是在大鼠体内造成显著心脏肥大的最低剂量。Lai 等，同上。一次小试显示，上述剂量的 IFN-γ抑制氟前列醇诱导的心脏肥大，而且对大鼠的体重几乎没有影响。

血流动力学评价

处理后第 13 天，腹膜内注射氯胺酮 80mg/kg(Aveco Co. Inc., Fort Dodge, Iowa)和赛拉嗪 10mg/kg(Rugby Laboratories, Inc., Rockville Center, NY)麻醉大鼠。在腹主动脉内植入装有肝素-盐溶液(50U/ml)的导管(PE-10 接 PE-50)，通过股动脉，用于测定平均动脉压(MAP)和心率(HR)。导管穿出固定于颈后。

插导管后 1 天，将动脉导管与 CP10 型压力传感器(Century Technology Company, Inglewood, CA, USA)连接，传感器与 Grass 7 型多种波动扫描器(Grass

Instruments. Quincy, MA, USA)连接。同时测定清醒的、非克制小鼠的 MAP 和 HR。

测定器官重量

氯胺酮/赛拉嗪麻醉下，取出心脏、肾脏和脾脏，解剖，称重。左心室保存于 80℃，用于评价基因表达。

压力超负荷动物模型

通过部分结扎大鼠腹主动脉诱导压力超负如现有技术所述。Kimura 等，Am. J. Physiol. 1989: 256(Heart Circ. Physiol.25):H1006-H1011；Batra 等，J. Cardiovasc. Pharmacol. 17(增刊 2), S151-S153(1991)。简而言之，如前所述用氯胺酮/赛拉嗪麻醉大鼠。在腹壁上造一 3mm 切口。露出隔膜与肾动脉之间的腹主动脉，用 5-0 号丝缝线结扎。将缝线绕一根 23 号针扎紧，然后抽出针。假处理组动物也接受手术，但不用缝线进行结扎。

压力超负荷大鼠内的试验方案

主动脉被结扎的大鼠随机地，术前 1 天，2 次皮下注射 0.08mg/kg IFN-γ，术后注射 14 天。假处理组动物不接受处理。处理后第 13 天，如前所述在隔膜下右颈动脉内植入一根导管。植入后 1 天，测定清醒大鼠的动脉压和 HR。取出心脏和肝脏、肾脏及脾脏等其它器官，称重，用 10%经缓冲的福尔马林固定，以备病理学研究之用。迅速切取部分动物的左心室，用液氮冷冻，保存于-80℃，以备基因表达研究之用。

统计学分析

将结果表示为平均值±SEM。进行单向方差分析(ANOVA)，测定参数的组间差异。然后用 Newman-Keuls 法对明显差异进行 post-hoc 分析；$p<0.05$ 被认为明显。

RNA 制备

用 RNeasy Maxi 柱(Qiagen)，按照生产商的说明，分离总 RNA。

RT-PCR

用实时 RT-PCR(TaqMan)技术比较不同处理组之间的基因表达差异。设计一寡核苷酸探针，3'端含荧光染料 6-羧基四甲基-罗丹明(TAMRA)，用它与两 PCR 引物界定的扩增子杂交。3'封端磷酸酯避免了探针的延长。在 PCR 反应的延长阶段，报道染料因 Taq 聚合酶的 5'外切活性从探针上释放。不再进一步处理，就在反应试管中用测序仪监测产生的荧光，并定量，因此称之为“实时”。阈值循环数(Ct)，即报道剂荧光超过基线标准偏差 10 倍以上的时候，与样品产生的复制子

量成正比。因为荧光是在扩增反应对数期测定的，所有反应组分都不成为限制。在各试验中，分析一个没有 RNA 模板的对照以监测污染，并包括另一个省略了 RT 步骤的对照以消除可能成为信号源的污染性 DNA 扩增。通过调节镁离子和引物浓度优化反应，以做到荧光信号最强，Ct 最小。产物在琼脂糖凝胶上电泳，确认在预计分子量处只有一条带。此外，在基因库中筛选扩增子的序列，消除与密切相关基因重叠的可能性。

对每份样品来说，如后文所述，用标准曲线测定每个靶基因的 mRNA，并相对样品内的甘油醛-3-磷酸酯-脱氢酶(GAPDH)含量进行归一(有关具体计算，参见后文)。然后，可比较处理组之间各靶基因相对 GAPDH 的相对丰度。

RT-PCR

每份反应取 1ng 总 RNA，用 TaqMan 7700 型测序仪(ABI-Perkin Elmer)进行 RT-PCR(Gibson 等，Genome Res. 6, 995-1001(1996))。(50μl)扩增反应的条件为：1X TaqMan 缓冲液 A，200μM dATP，dCTP，dGTP 和 400μM dUTP，10%甘油，6.5mM $MgCl_2$，50U MuLV 逆转录酶，20U Rnase 抑制剂，1.25U AmpliTaq Gold，100nM 正向引物和反向引物，100nM 荧光探针。RT-PCR 试剂和甘油分别购自 Perkin Elmer 和 Sigma。在 MicoAmp 光学管和盖子(ABI-Perkin Elmer)中进行反应。根据 Perkin Elmer 确定的方针设计 TaqMan 引物，在 Genentech Inc.合成，鼠 GAPDH 的探针则是 Perkin Elmer 赠送的。逆转录在 48℃进行 30 分钟，然后在 95℃进行 10 分钟的 AmpliTaq 金的活化。热循环为 95℃30 秒，60℃1.5 分钟，共 40 轮。

如 Heid 等，Genome Res. 6:986-994(1996)所述，修改后进行 TaqMan 结果的定量。简而言之，对各感兴趣基因的标准曲线(1:5 连续稀释)测定 2 次。以 Ct 为 Y 轴，以总 RNA 浓度的 Log 值为 X 轴，确定描述该曲线的方程。根据相应的标准曲线，代入 Ct(Y 值)，求 mRNA(X)，测定各靶基因的 mRNA。然后，用以下方程将各靶基因的值相对 GAPDH 归一：$10^{X1}/10^{X2}$，其中，X1 是靶基因，X2 是 GAPDH。

结果

IFN-γ在体内抑制心脏肥大

已知，长期给予 $PGF_{2\alpha}$的活化剂类似物氟前列醇在体内诱导心脏肥大，而且，患心肌梗塞所致病理性心肌肥大的大鼠其心肌内可抽提 $PGF_{2\alpha}$水平已被逐步提高(Lai 等，同上)。因此，能在体内抑制 $PGF_{2\alpha}$对心肌生长作用的因子也许可用于治

疗心肌肥大。给予或不给予 IFN-γ的情况下给予氟前列醇，持续 2 周，测定对心脏肥大的影响。与载体对照相比，在氟前列醇处理的大鼠中，心脏、心室和左心室的绝对重量倾向于增加，但与氟前列醇处理的大鼠相比，用氟前列醇+ IFN-γ处理的大鼠体内，以上重量明显减小(表 1)。氟前列醇处理造成心脏、心室和左心室重量与体重(BW)之比明显增大，说明氟前列醇诱导心脏肥大(图 4)。IFN-γ抑制氟前列醇诱导的肥大。与氟前列醇组相比，接受氟前列醇+ IFN-γ的大鼠其心脏、心室和左心室比 BW 的相对重量明显减轻(图 4)。IFN-γ组与载体组的比较显示，单用 IFN-γ对心脏、心室或左心室的绝对重量或 BW 相对重量没有明显改变(表 1，图 4)。

长期给予氟前列醇与平均动脉压(MAP)相比载体处理组的显著降低相关(图 5)。与载体相比，IFN-γ对 MAP 没有作用，而且对氟前列醇处理动物的 MAP 也没有影响。4 个处理组的心率有明显改变(图 5)。以上结果说明，IFN-γ并不通过抵消氟前列醇的血流动力学作用来抑制其诱导的心脏肥大。

IFN-γ不仅抑制与使用氟前列醇相关的心脏重大，而且改变与氟前列醇诱导的肥大相关的心内基因表达(图 6)。与载体相比，氟前列醇处理大鼠心脏内，α-骨骼肌动蛋白、胶原 1 和利钠因子的 mRNA 丰度增加。这些大鼠中的肌质网钙 ATPase 显著减少。IFN-γ抑制除利钠因子反应之外的全部反应。

还在一腹主动脉结扎造成压力超负荷导致心脏肥大的鼠模型中进行了 IFN-γ试验。主动脉狭窄造成心脏肥大表现为心脏、心房、心室和左心室绝对重量以及与 BW 的相对重量的明显增加。IFN-γ处理显著减轻了该模型中的心脏肥大(表 2，图 7 和 8)。

还检查了 IFN-γ对其它器官的作用(表 2)。主动脉结扎和 IFN-γ处理都不改变肾脏的重量及其与 BW 之比。与假手术动物相比，载体处理并结扎主动脉的大鼠的肝脏重量及其与 BW 之比倾向于减小，用 IFN-γ处理的则不减小。主动脉结扎造成脾脏绝对重量和 BW 归一化重量的显著升高，IFN-γ处理更加剧这种升高。因此，IFN-γ对心脏肥大的作用并不是因为对器官重量的普遍作用。

主动脉结扎大鼠的平均动脉压、心收缩压和舒张压明显高于假手术对照，而且，IFN-γ处理与载体处理的结扎大鼠之间，动脉压的增加没有区别(图 9)。该结果说明，接受 IFN-γ的结扎大鼠的心脏肥大缓解与后负荷改变无关。

主动脉结扎造成部分心内基因表达的改变。与假手术对照相比，结扎大鼠内，β-肌球蛋白重链、α-平滑肌肌动蛋白、α-骨骼肌动蛋白、心房利钠因子、胶原 1 和 III 和纤连蛋白的 mRNA 相对丰度都上升。3 个基因中的 2 个：α-平滑肌

肌动蛋白和胶原 1 被 IFN-γ抑制(表 3)。

综上所述，实施例 1 和 2 的结果显示，IFN-γ能够抑制心脏肥大。IFN-γ的作用不仅仅在于抑制肥大刺激诱导的心脏增大，它还在基因表达水平抑制肥大心脏中发生的某些分子改变。特别值得指出的是，在对氟前列醇长期刺激的反应中，和在压力超负荷诱导的肥大模型中，IFN-γ都体内抑制胶原 1 基因的表达。心肌胶原中约 75%为胶原 I(Ju 等，Can. J. Caridol. 12:1259-1267(1996))。伴随心脏肥大的胞外基质沉淀增加和间质纤维化可能是因为心力衰竭的病理生理学。通过抑制胶原 1 的产生，IFN-γ能减轻心力衰竭中的间质纤维化。

表 1. Flup 和/或 IFN 处理的大鼠的体重和器官重量

	体重	Flup	Flup+IFN	IFN
BWO(g)	292.4±1.7	292.3±2.2	292.8±2.1	292.5±3.2
BW(g)	391.6±6.3	381.1±4.4	377.6±4.5	380.8±6.1
$_{\Delta}$BW(g)	99.2±5.5	91.9±4.0	84.8±3.9	88.3±5.0
HW(g)	0.966±0.22	1.000±0.018	0.9279±0.016#	0.956±0.029
VW(g)	0.922±0.22	0.957±0.017	0.889±0.015#	0.914±0.029
LVW(g)	0.706±0.018	0.740±0.013	0.678±0.011#	0.696±0.023
KW(g)	1.440±0.035	1.397±0.038	1.377±0.031	1.327±0.035
KW/BW(g/kg)	3.678±0.075	3.632±0.076	3.648±0.070	3.483±0.069
SW(g)	0.799±0.050	0.880±0.048	1.009±0.042*	0.924±0.068
SW/BW(g/kg)	2.065±0.149	2.309±0.130	2.676±0.082**	2.415±0.149

所给的数据是平均值±SEM，载体、Flup、Flup+IFN 和 IFN 组的动物数量分别是 14、14、14 和 9。载体：盐水；Flup：氟前列醇；IFN：干扰素γ；BWO：基础体重；BW：处理后体重；$_{\Delta}$BW：BW-BWO；HW：心脏重量；VW：心室重量；LVW：左心室重量；KW：肾脏重量；SW：脾脏重量。*p<0.05，**p<0.01，与载体组相比；#p<0.05，##p<0.01，与 Flup 组相比。

表 2. 压力超负荷大鼠的体重、器官重量和 HR

	假手术	PO+载体	PO+IFN
BWO(g)	278.8±1.9	279.4±1.3	279.0±1.3
BW(g)	367.9±6.8	347.5±5.7*	355.5±5.2
$_{\Delta}$BW(g)	89.1±5.8	68.1±5.6*	76.5±4.7
AW(g)	0.038±0.02	0.056±0.002**	0.046±0.003*##

AW/BW(g)	0.104±0.04	0.162±0.006**	0.129±0.007*##
KW(g)	1.438±0.51	1.334±0.033	1.349±0.071
KW/BW(g/kg)	3.894±0.078	3.841±0.070	3.776±0.071
LW(g)	13.84±0.55	12.53±0.36	13.96±0.47#
LW/BW(g/kg)	37.46±0.96	36.01±0.71	38.94±0.92#
SW(g)	0.724±0.030	0.839±0.026*	1.170±0.053**##
SW/BW(g/kg)	1.959±0.051	2.418±0.069**	3.261±0.121**##
HR(bpm)	371±12	415±12*	418±19*

所给的数据是平均值±SEM，测定所有参数时，假手术、PO+载体和 PO+IFN 组的动物数量分别是 16、22 和 21，测定 HR 时的动物数量为 7、8 和 7。PO：压力超负荷；IFN：干扰素γ；BWO：基础体重；BW：处理后体重；$_{\Delta}$BW：BW-BWO；AW：心房重量；KW：肾脏重量；LW：肝脏重量；SW：脾脏重量；HR：效率。*p<0.05，**p<0.01，与假手术组相比；#p<0.05，##p<0.01，与 PO+载体组相比。

表 3. IFN 对基因表达的影响

处理	假手术+载体	PO+载体	PO+IFN
ANF	0.98±0.37	5.29±1.21*	3.61±1.32
βMHC	0.89±0.24	1.91±0.15*	1.71±0.14*
SKA	0.95±0.13	3.35±0.46*	2.47±0.51*
SMA	0.71±0.06	0.89±0.04*	0.77±0.06
COLI	0.55±0.05	0.91±0.09*	0.77±0.10
COLIII	0.44±0.05	0.66±0.08*	0.72±0.09*
FIB	0.66±0.17	1.03±0.11*	0.97±0.12

PO：压力超负荷；IFN：干扰素γ；ANF：心房利钠因子；βMHC：β肌球蛋白重链；SKA：α骨骼肌动蛋白；SMA：α平滑肌肌动蛋白；COLI：胶原 I；COLIII：胶原 III；FIB：纤连蛋白。表达水平计算为与甘油醛-3-磷酸酯脱氢酶之比。每组的 n=6。数据是平均值±SEM。*与假手术+载体组相比的 p<0.05。

说 明 书 附 图

图 1A

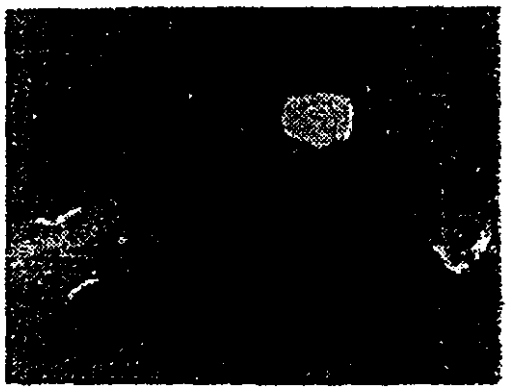


图 1B

图 1C

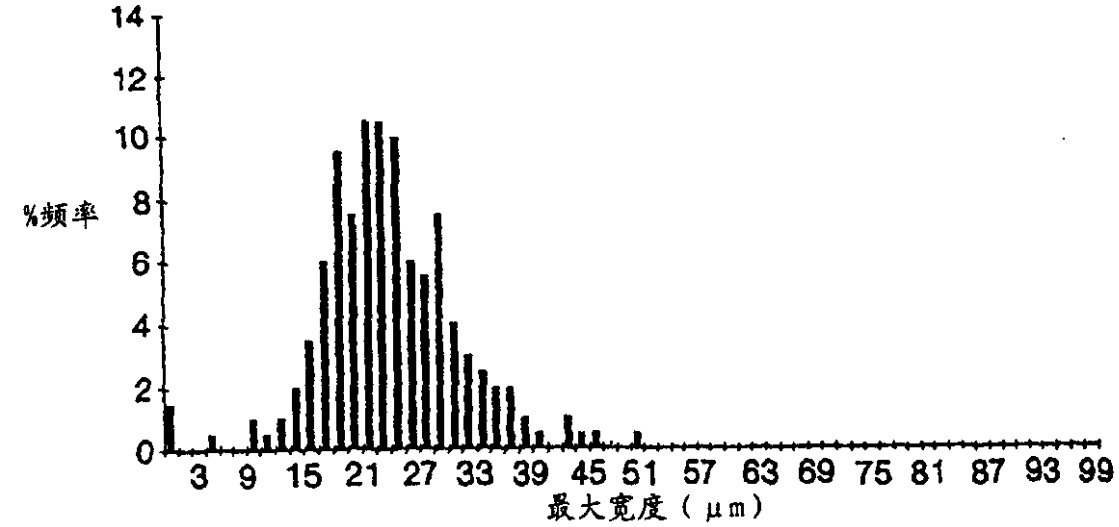

%频率
0
2
4
6
8
10
12
14
3 9 15 21 27 33 39 45 51 57 63 69 75 81 87 93 99
最大宽度（μm）

图 1D

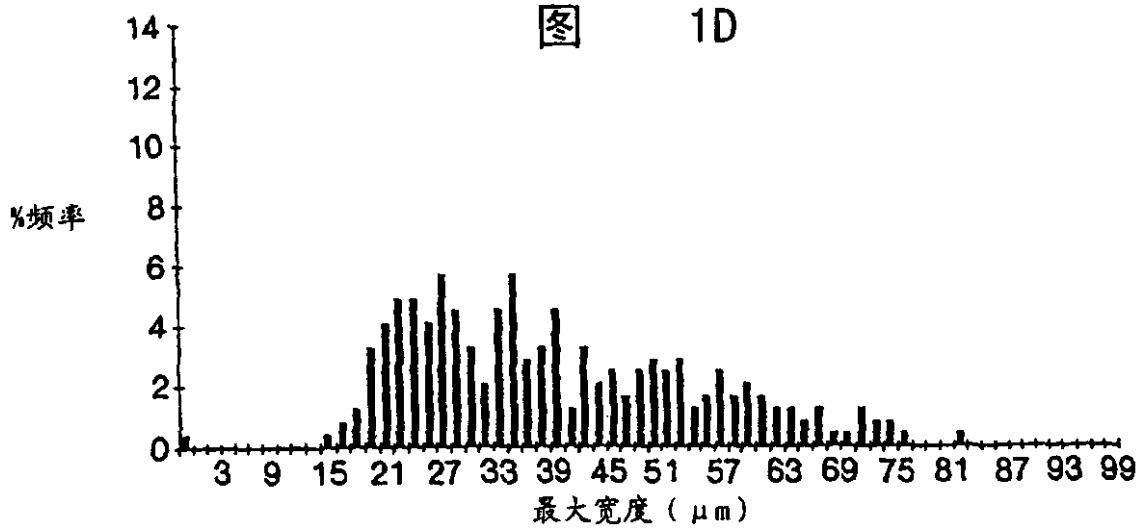

%频率
0
2
4
6
8
10
12
14
3 9 15 21 27 33 39 45 51 57 63 69 75 81 87 93 99
最大宽度（μm）

图 1E

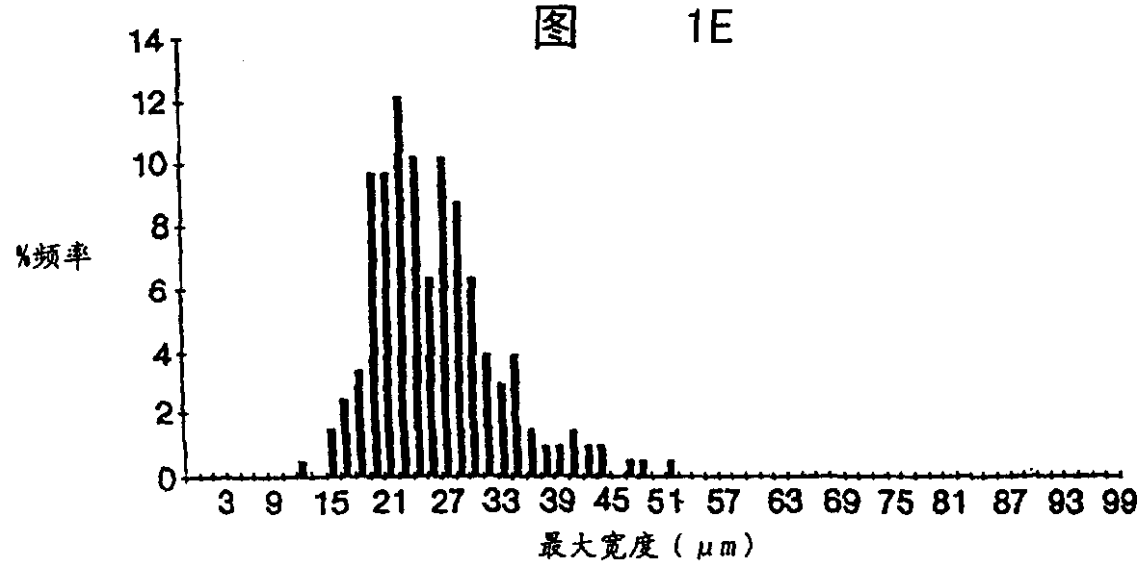

%频率
0
2
4
6
8
10
12
14
3 9 15 21 27 33 39 45 51 57 63 69 75 81 87 93 99
最大宽度（μm）

图 1F

图 2A

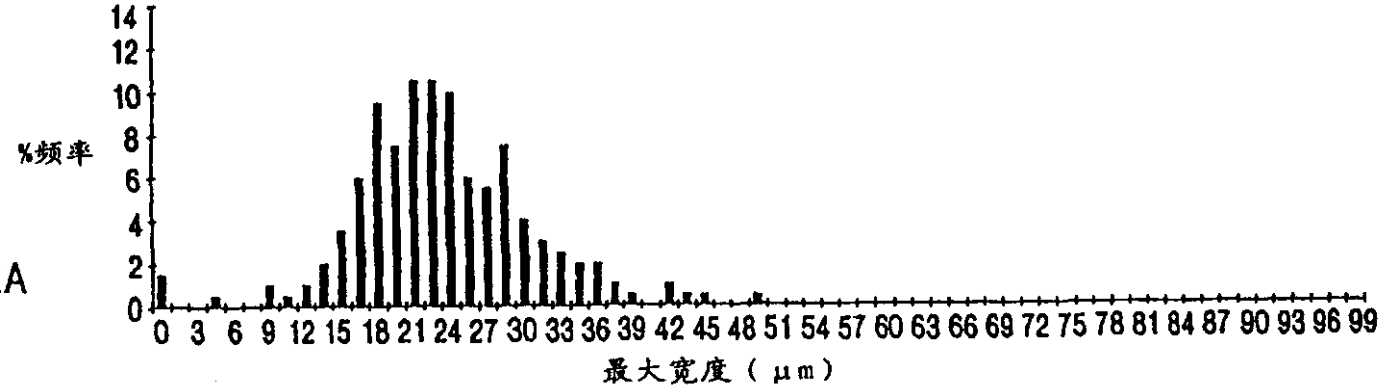

%频率
14
12
10
8
6
4
2
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99
最大宽度（μm）

图 2B

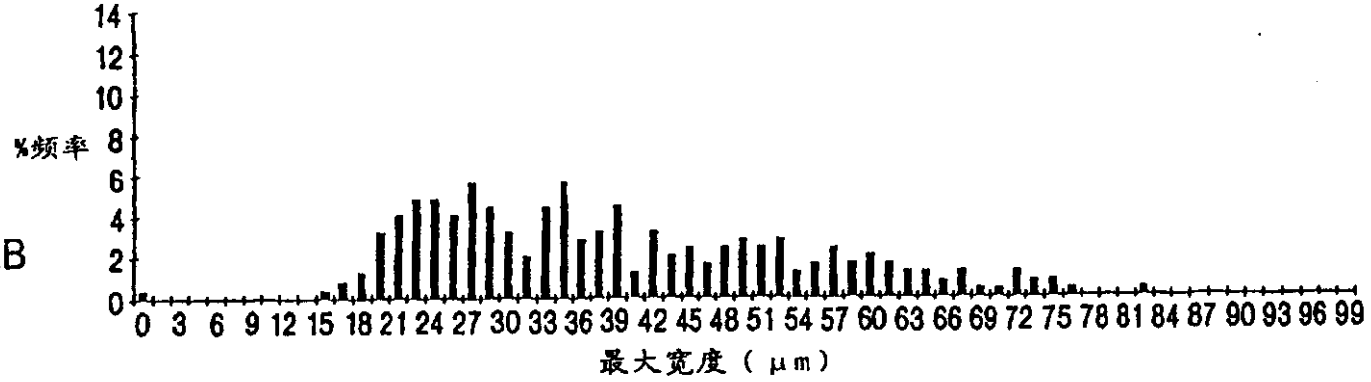

%频率
14
12
10
8
6
4
2
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99
最大宽度（μm）

图 2C

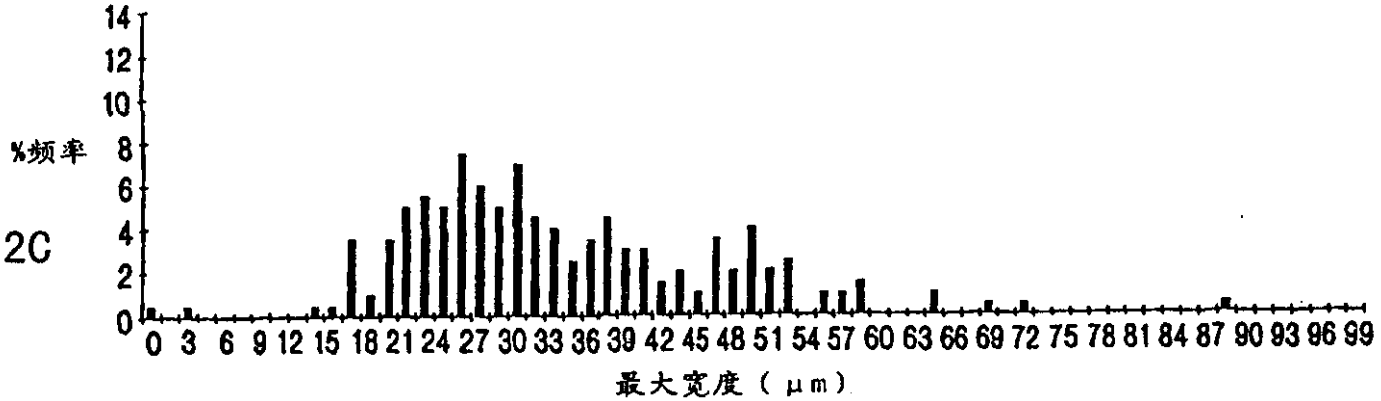

%频率
14
12
10
8
6
4
2
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99
最大宽度（μm）

图 2D

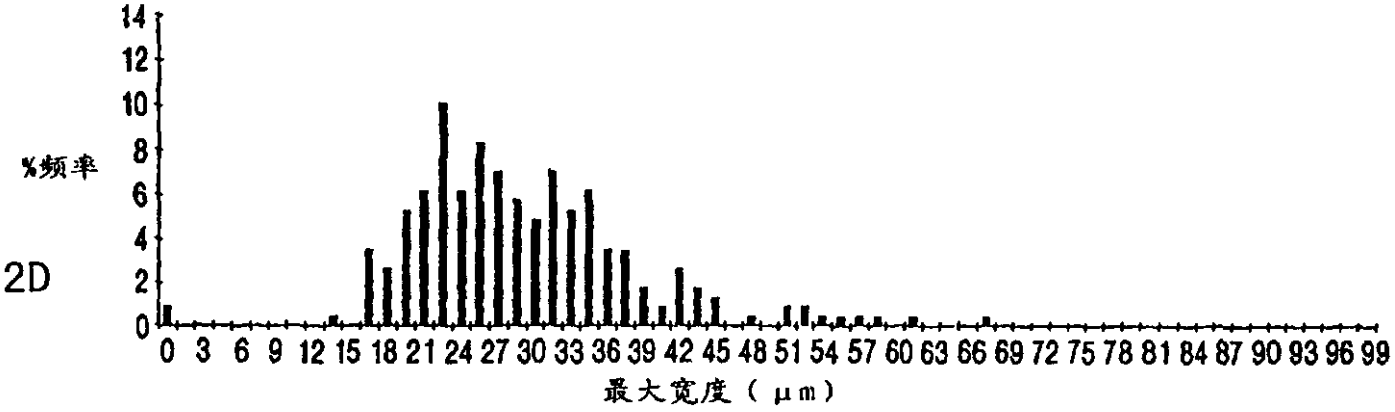

%频率
14
12
10
8
6
4
2
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99
最大宽度（μm）

图 2E

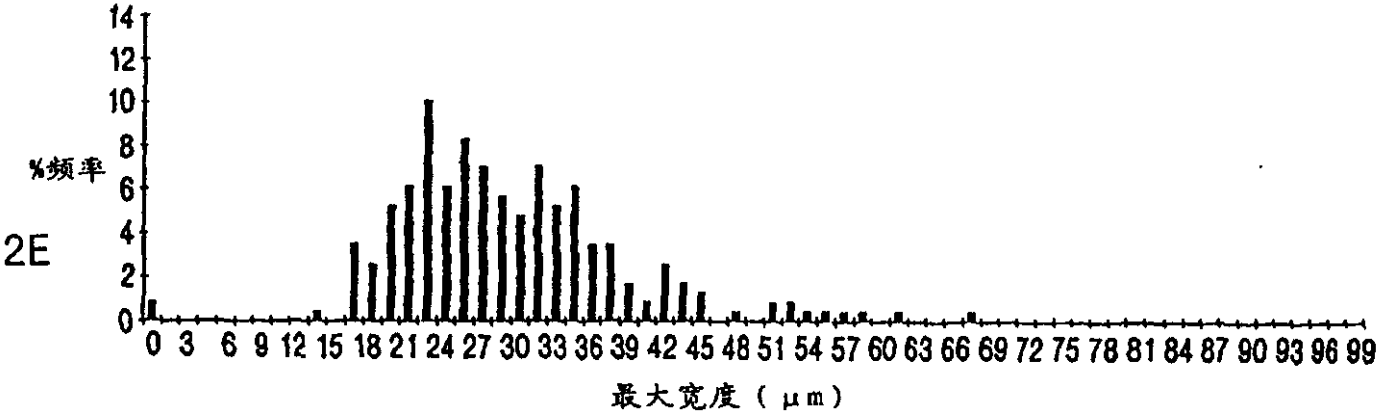

%频率
14
12
10
8
6
4
2
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99
最大宽度（μm）

图 3A

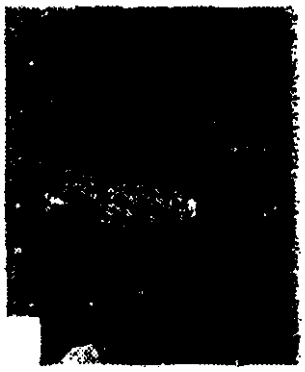


图 3B

图 3C

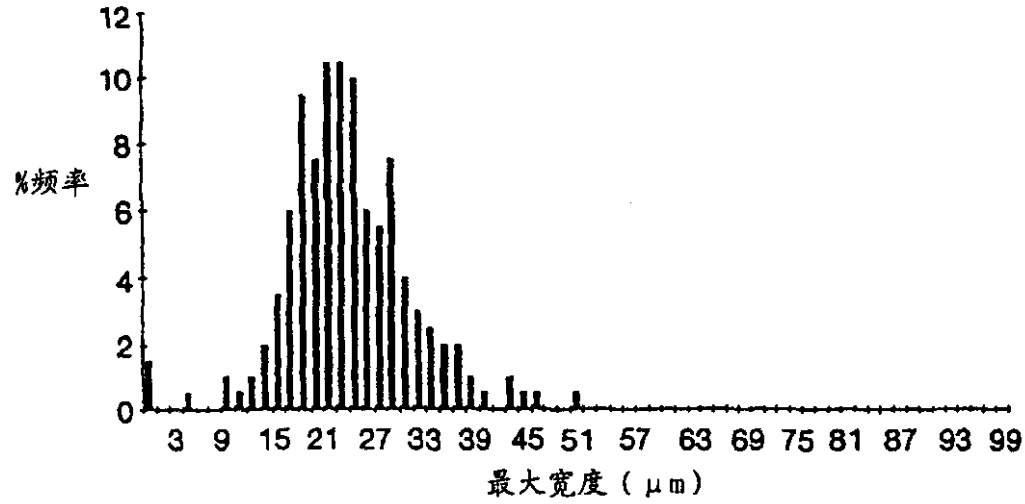

%频率
12
10
8
6
4
2
0
3 9 15 21 27 33 39 45 51 57 63 69 75 81 87 93 99
最大宽度（μm）

图 3D

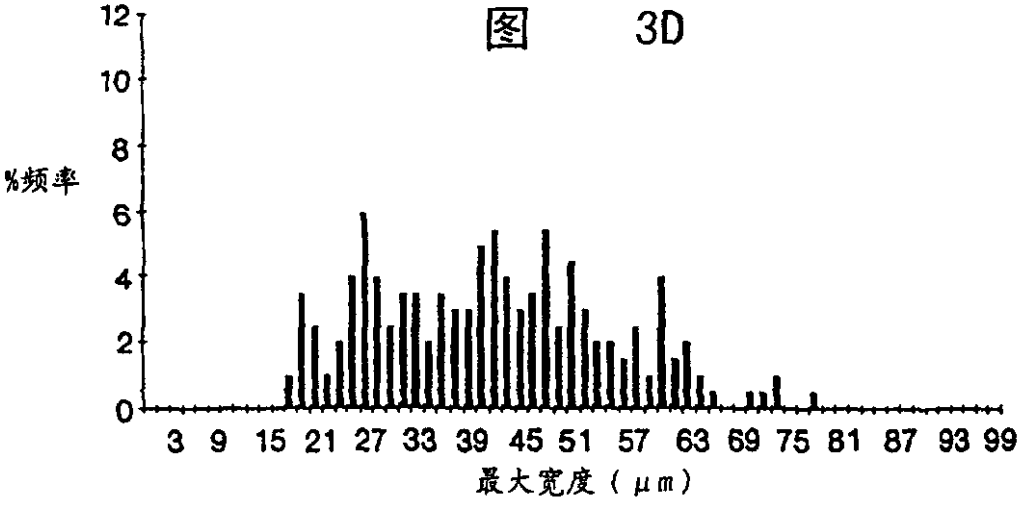

%频率
12
10
8
6
4
2
0
3 9 15 21 27 33 39 45 51 57 63 69 75 81 87 93 99
最大宽度（μm）

图 3E

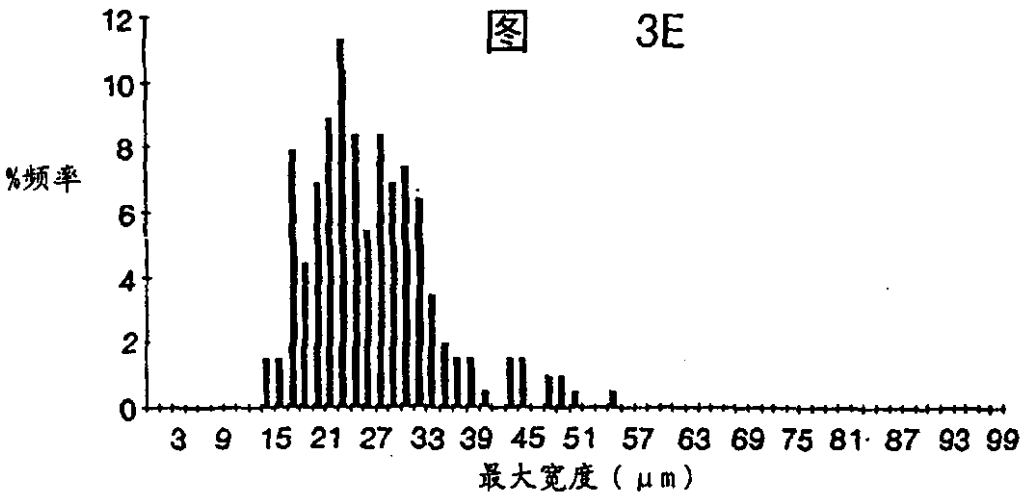

%频率
12
10
8
6
4
2
0
3 9 15 21 27 33 39 45 51 57 63 69 75 81 87 93 99
最大宽度（μm）

图 3F

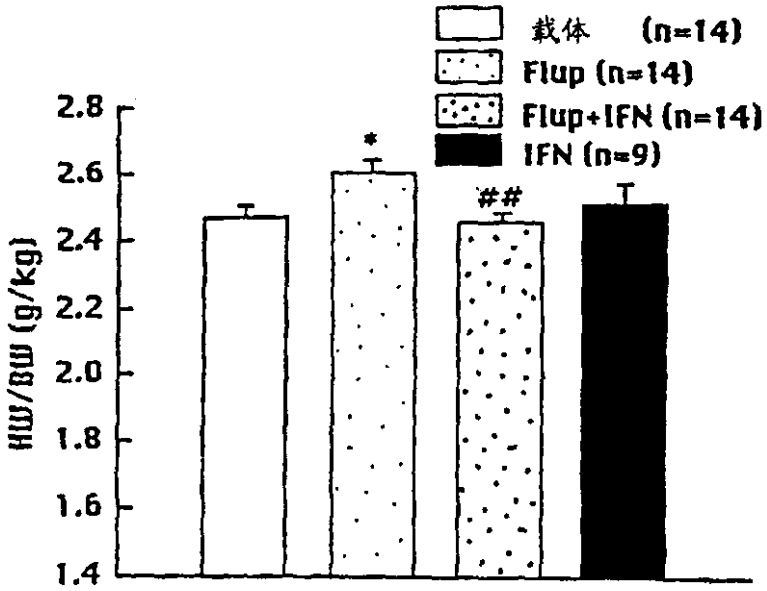

载体 (n=14)
Flup (n=14)
Flup+IFN (n=14)
IFN (n=9)
HW/BW (g/kg)
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
*
##

图 4A

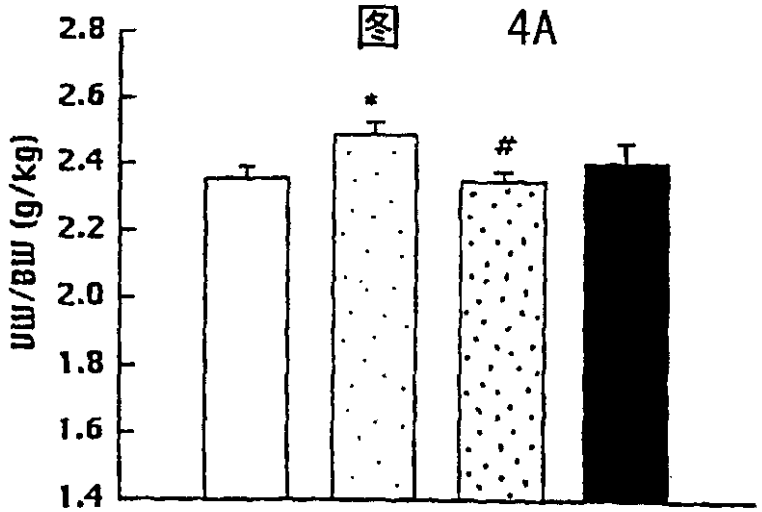

LW/BW (g/kg)
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
*
#

图 4B

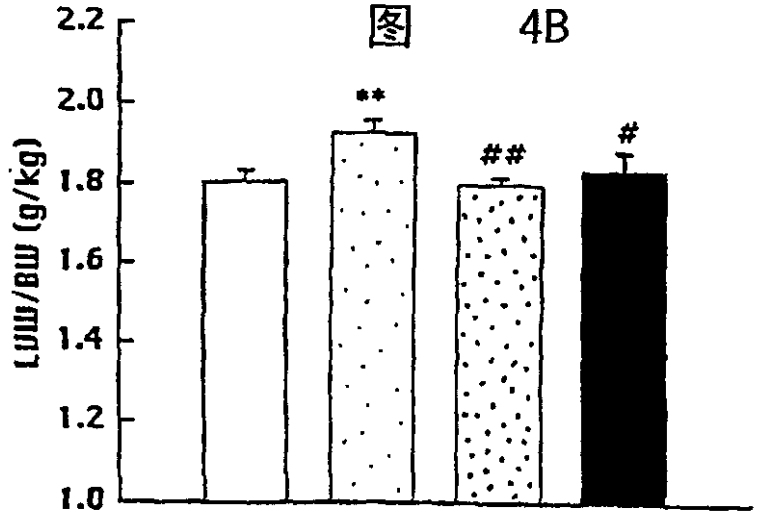

LuW/BW (g/kg)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
**
##
#

图 4C

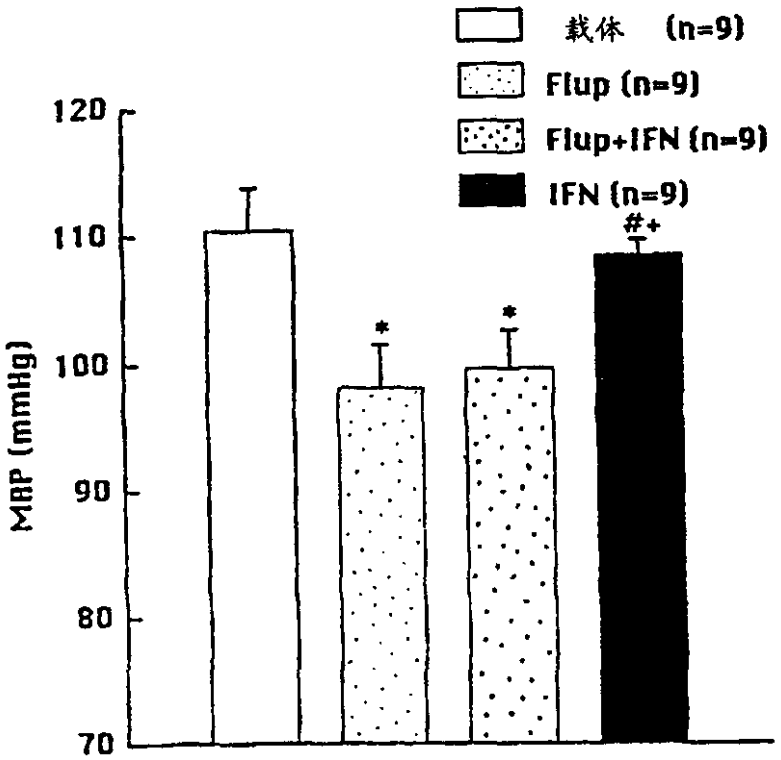

载体 (n=9)
Flup (n=9)
Flup+IFN (n=9)
IFN (n=9)
MAP (mmHg)
120
110
100
90
80
70
*
*
#+

图 5A

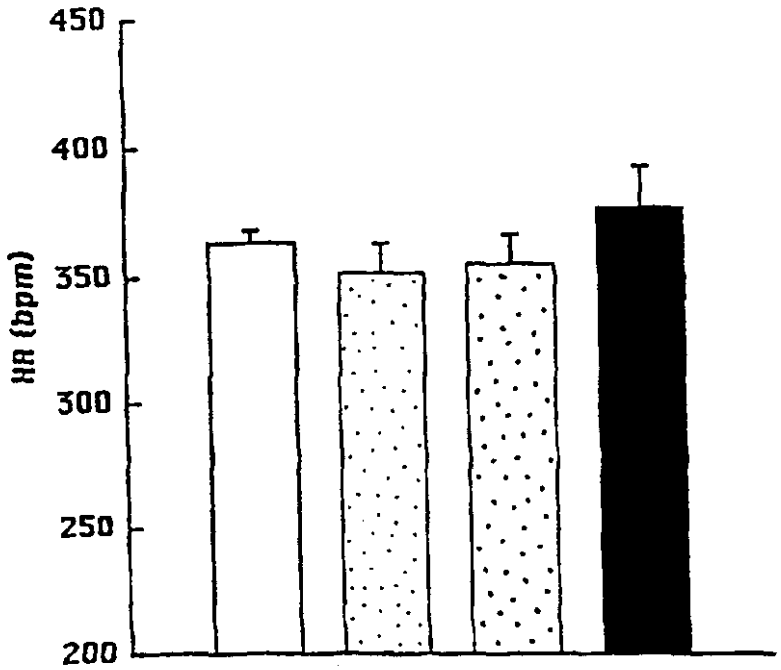

HR (bpm)
450
400
350
300
250
200

图 5B

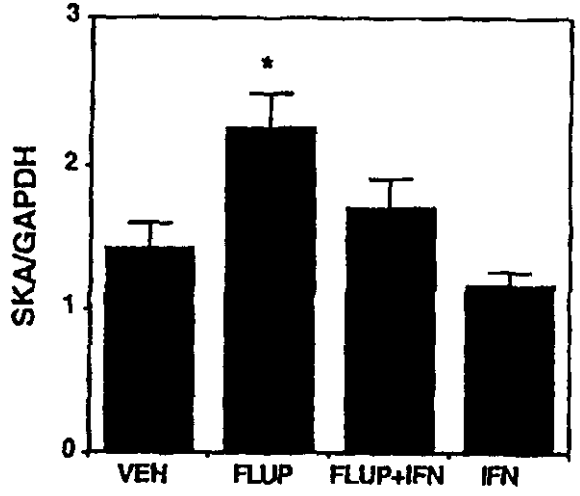


图 6A

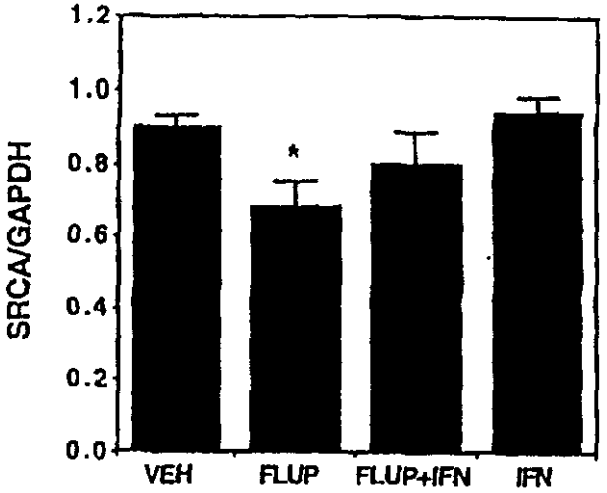


图 6B

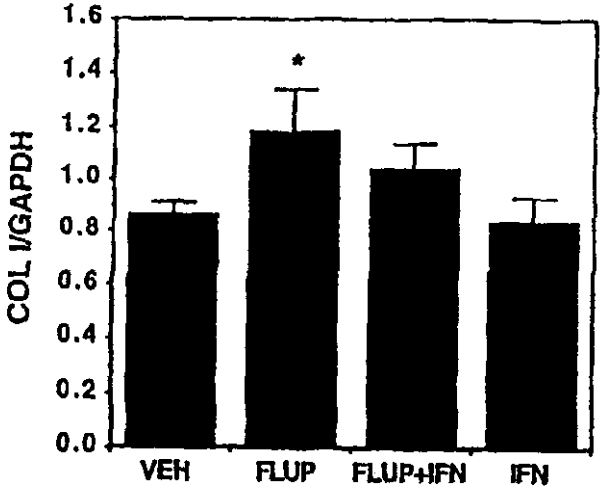


图 6C

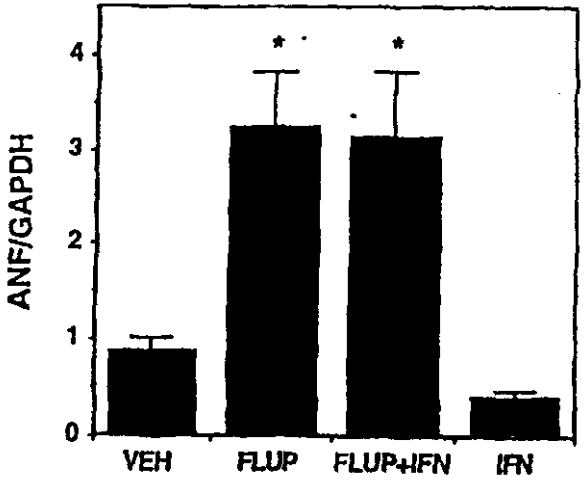


图 6D

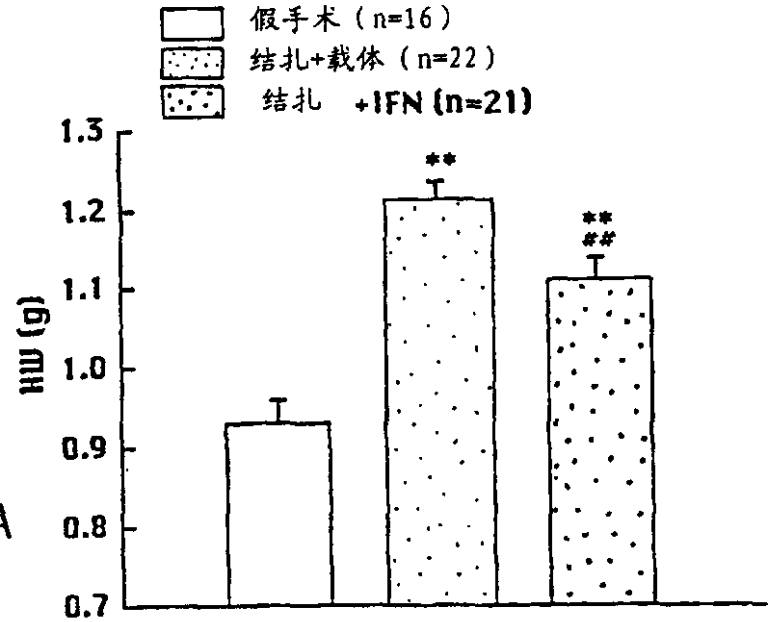

假手术（n=16）
结扎+载体（n=22）
结扎 +IFN (n=21)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
**
**
##

图 7A

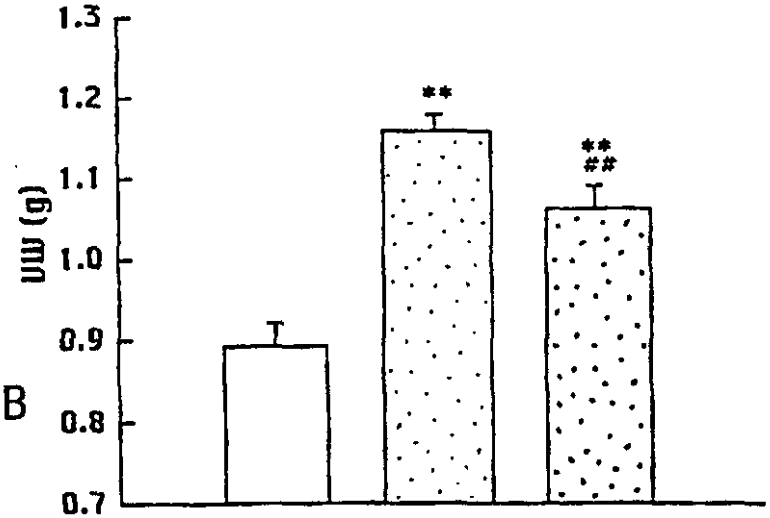

1.3
1.2
1.1
1.0
0.9
0.8
0.7
**
**
##

图 7B

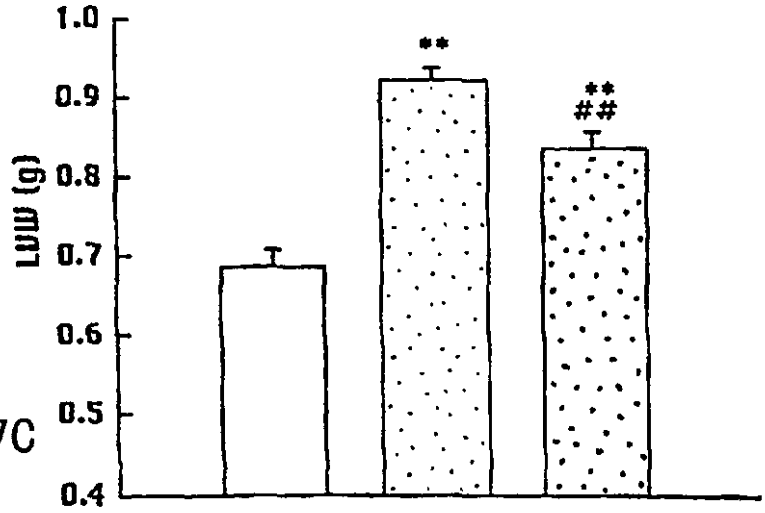

1.0
0.9
0.8
0.7
0.6
0.5
0.4
**
**
##

图 7C

图 8A

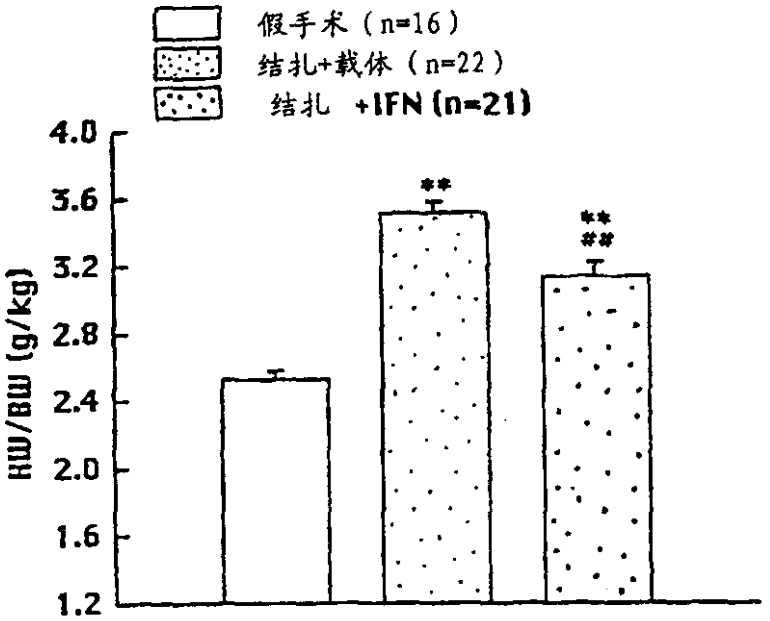

假手术（n=16）
结扎+载体（n=22）
结扎 +IFN (n=21)
HW/BW (g/kg)
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
**
**
##

图 8B

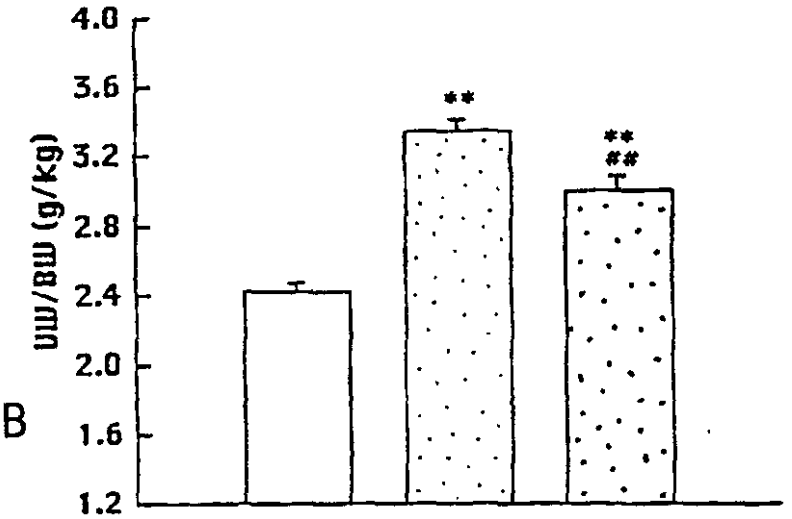
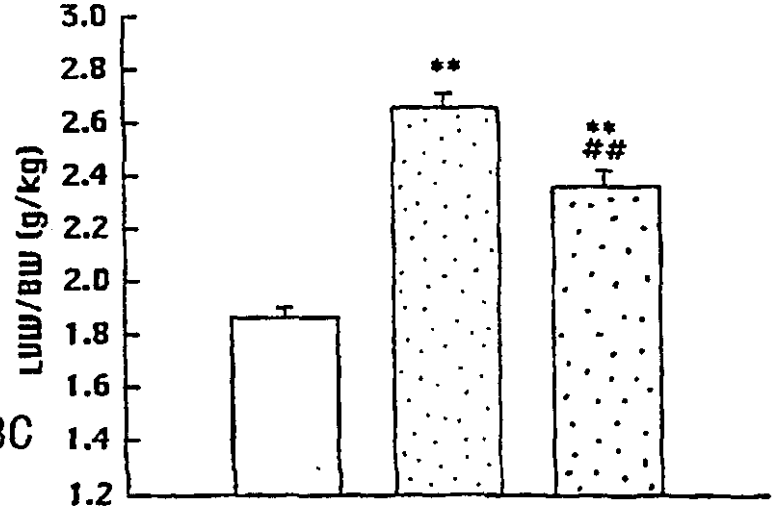

UW/BW (g/kg)
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
**
**
##

图 8C


LUW/BW (g/kg)
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
**
**
##

00.10.06

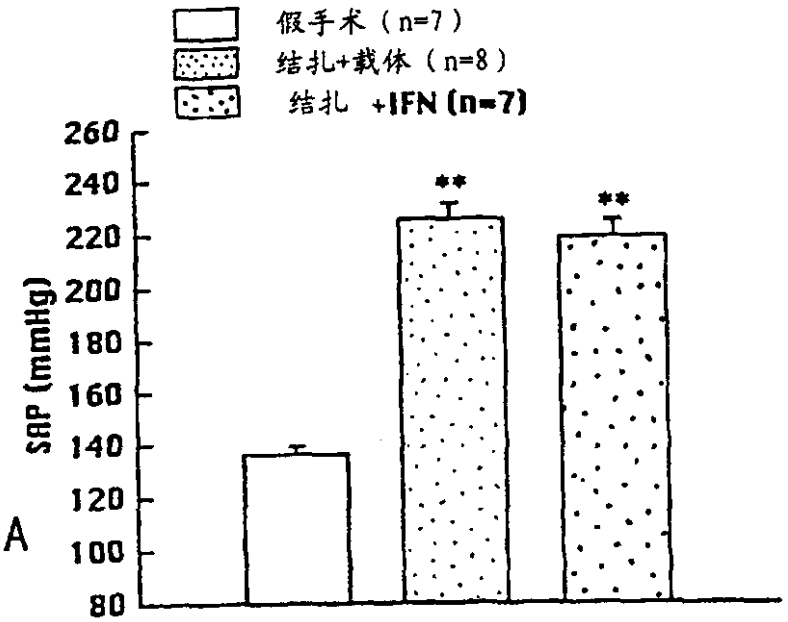


图 9A

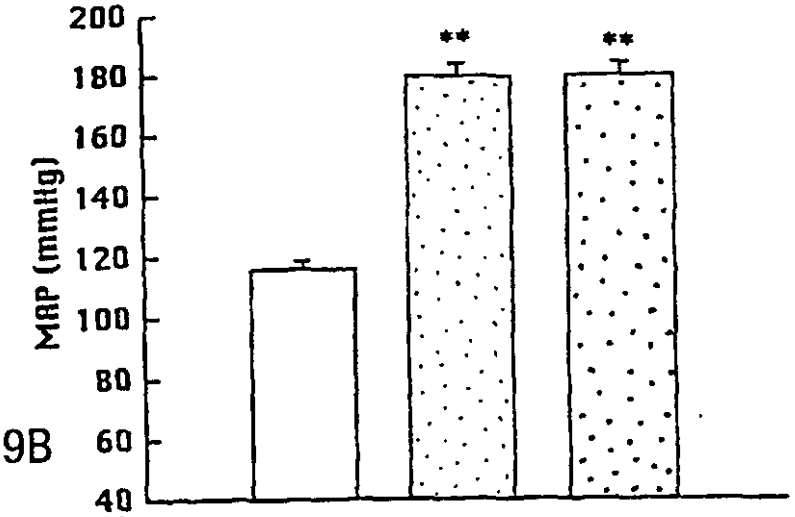


图 9B

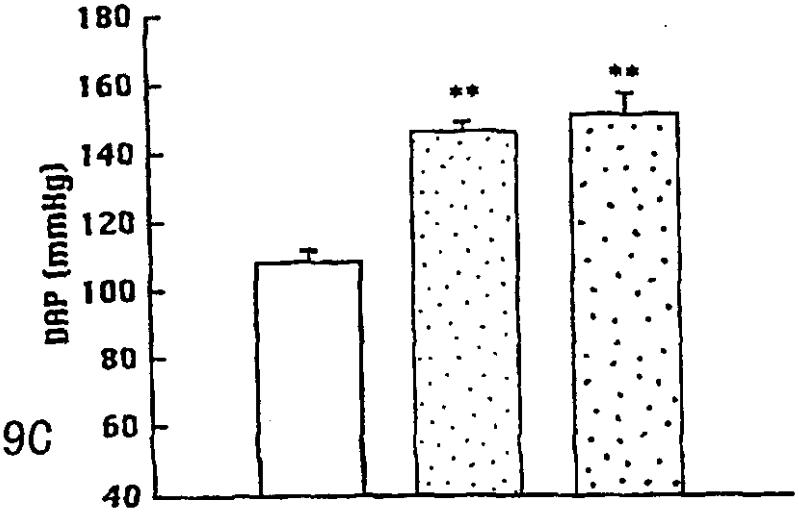


图 9C