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**(54) Title:** cDNA CLONE FOR HUMAN INDUCIBLE NITRIC OXYDE SYNTHASE AND PROCESS FOR PREPARING SAME

**(57) Abstract**

A human tissue inducible nitric oxyde synthase cDNA clone is disclosed. A process for preparing this cDNA clone coding for human tissue inducible nitric oxyde synthase and for expressing the human tissue inducible nitric oxyde synthase protein are provided.

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# cDNA CLONE FOR HUMAN INDUCIBLE NITRIC OXIDE SYNTHASE AND PROCESS FOR PREPARING SAME

## Background of the Invention

The invention described herein was made in the course of work supported in part by Public Health Service, Grant Nos. GM44100 and GM37753 from the National Institutes of Health, General Medical Sciences.

The following microorganisms have been deposited by David A. Geller on behalf of the University of Pittsburgh of the Commonwealth System of Higher Education, Pittsburgh, Pennsylvania 15260, USA, on November 18, 1992, with and are available from the permanent collection of the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 202852-1776, USA:

| | |
|---|---|
| ATCC 75358 | Human Hepatocyte Inducible Nitric Oxide Synthase cDNA in pBluescript (pHINOS) |
| ATCC 69126 | Human Hepatocyte Inducible Nitric Oxide Synthase cDNA in pBluescript transformed in E. coli SOLR bacteria (plasmid HINOS cDNA) |

The American Type Culture Collection has performed viability tests on each of the hereinbefore mentioned deposited microorganisms and has concluded on November 20, 1992, that each of the hereinbefore mentioned deposited microorganisms is viable and capable of reproduction.

These deposits are available to the public upon the grant of a patent to the assignee, the University of Pittsburgh of the Commonwealth System of Higher Education, disclosing them. However, it should be understood that the availability of these deposits does not constitute a license to practice this invention in derogation of patent rights granted by governmental action.

Field of the Invention

This invention relates to a human tissue inducible nitric oxide synthase cDNA clone capable of expressing a human inducible nitric oxide synthase protein, and a process suitable for cloning a cDNA encoding amino acid sequences for the human inducible nitric oxide synthase. More specifically, this invention relates to a human hepatocyte inducible nitric oxide synthase cDNA clone and to a process for cloning and expression of the human hepatocyte inducible nitric oxide synthase cDNA to provide a source of the human hepatocyte inducible nitric oxide synthase enzyme.

This invention provides a process for cloning a cDNA having an amino acid sequence coding for the human hepatocyte inducible nitric oxide synthase. Figures 1A-G show and SEQ ID NO: 1 in the SEQUENCE LISTING contains the 4,145 nucleotide bases for the sense strand of cDNA for human hepatocyte inducible nitric oxide synthase and sets forth the base codes as triplets (codon) for the coding parts of the nucleotide sequence. Figures 1A-G show and SEQ ID NO: 1 sets forth the amino acid sequence for the cDNA clone for human hepatocyte inducible nitric oxide synthase encoding amino acids 1 through 1153 of the human hepatocyte inducible nitric oxide synthase enzyme.

## BRIEF DESCRIPTION OF BACKGROUND ART

It is known by those skilled in the art that nitric oxide (NO) is a biologic mediator derived from the amino acid L-arginine. One of a family of enzymes, nitric oxide synthase (NOS), acts upon L-arginine to oxidize one of the guanidino nitrogens to NO while citrulline is formed from the remainder of the L-arginine molecule. Nitric oxide is a very short-lived free radical and is rapidly oxidized to nitrite ($NO_2$-) and nitrate ($NO_3$-) which is measured as the stable inactive end products of nitric oxide formation.

It is well known by those skilled in the art that multiple isoforms of the nitric oxide synthase enzyme exist and that they are generally classified into two broad categories:

1) constitutive and 2) inducible. These classes of NOS enzymes vary considerably in their size, amino acid sequence, activity and regulation. For example, cells such as neurons and vascular endothelial cells contain constitutive NOS isotypes while macrophages and vascular smooth muscle cells express an inducible NOS.

It is generally well known that small amounts of NO generated by a constitutive NOS appear to act as a messenger molecule by activating soluble guanylate cyclase and, thus, increasing intracellular guanosine, 3′, 5′ - cyclic monophosphate (cGMP) and the induction of biological responses that are dependent on cGMP as a secondary messenger. For example, through this mechanism, endothelial derived NO induces relaxation of vascular smooth muscle and is identified as endothelium derived relaxing factor (EDRF). Nature, Vol. 327, pp. 524-526 (1987) and Proc Natl Acad Sci USA, Vol. 84, pp. 9265-9269 (1987). Another example includes, but is not limited by, neuronal nitric oxide which acts as a neuro transmitter by activating guanylate cyclase with important functions in the central nervous system and autonomic nervous systems. Proc Natl Acad Sci USA, Vol. 86, pp. 9030-9033 (1989) and Science, Vol. 257, p. 401 (1992).

It is generally known by those skilled in the art that the larger quantities of nitric oxide produced by the inducible nitric oxide synthase have antimicrobial and antitumor functions. J. Clin. Invest., Vol. 81, pp. 1129-1136 (1989) and Science, Vol. 235, pp. 473-476 (1987), respectively. It is also known by those skilled in the art that when vascular smooth muscle cells are stimulated to express a NOS enzyme by inflammatory cytokines, the excess amounts of nitric oxides that are produced contribute to the vascular collapse seen in sepsis. FEBS Lett., Vol. 265, pp. 133-136, (1990).

Thus, it will be appreciated that nitric oxide has both normal physiologic intracellular and extracellular regulatory functions. However, excessive production of nitric oxide is detrimental. For example, stimulation of inducible nitric oxide

synthesis in blood vessels by bacterial endotoxin such as for example bacterial lipopolysaccharide (LPS) and cytokines that are elevated in sepsis results in massive dilation of blood vessels and sustained hypotension commonly encountered in septic shock. Proc. Natl. Acad. Sci USA, Vol. 87, pp. 3629-32 (1990). It is known that overproduction of nitric oxide in the lungs stimulated by immune complexes directly damages the lung. J. Immunol., Vol. 148, p. 3086 (1992). Induction of nitric oxide synthase in pancreatic islets impairs insulin secretion and contributes to the onset of juvenile diabetes. J. Biol. Chem., Vol. 266, p. 21351 (1991).

It will be appreciated that there is a great need in the medical community for collective inhibition of the inducible form of NOS but not the constitutive types of NOS in humans because this would allow for a means of preventing, such as for example, the hypotensive shock seen in sepsis, without preventing the physiologic regulation of vasomotor tone or neuro transmission in the central nervous system.

We recently demonstrated that nitric oxide biosynthesis is induced in isolated human hepatocytes after stimulation with interleukin-1, tumor necrosis factor-alpha, interferon-gamma and bacterial lipopolysacharride (bacterial endotoxin). FASEB JOURNAL, Vol. 6, No. 5, page A1834 (April, 1992) and J. Exp. Med., Vol. 176, p. 261 (1992). Heretofore no human cell type was known to show increased production of nitrogen oxides when treated with cytokines. Res. Immunol., Vol. 142, p. 557 (1991). It is generally known by those skilled in the art that all attempts to induce nitric oxide synthase in human macrophages and related cells typical to those found in rodent macrophages have failed. Res. Immunol., Vol. 142, p. 562, 589-90 (1991).

In spite of this background material, there remains a very real and substantial need for a cDNA clone for human tissue inducible nitric oxide synthase and a process for the molecular cloning of the same.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-G show the cDNA sense sequence (top line of each horizontal row) and the amino acid sequence of amino acids 1-1153 (bottom line of each horizontal row) for the cDNA clone for human hepatocyte inducible nitric oxide synthase, SEQ ID NO:1.

Figure 2 shows a Northern Blot of a mouse macrophage NOS cDNA cross-hybridizing to human hepatocyte (HC) nitric oxide synthase mRNA.

Figure 3 shows a Northern Blot of induced nitric oxide synthase mRNA isolated from three separate human liver samples using mouse macrophage cDNA.

Figure 4 shows a Northern Blot of poly A mRNA purified from 2 separate human liver samples for the construction of the cDNA library for isolation of the cDNA clone for the human hepatocyte inducible nitric oxide synthase.

Figure 5 shows a Northern Blot using cDNA isolated from human hepatocytes that sets forth the time course of induction of human nitric oxide synthase mRNA following cytokine and LPS stimulation.

## SUMMARY OF THE INVENTION

The present invention has met the hereinbefore described needs. The present invention provides a cDNA clone for human tissue inducible nitric oxide synthase and a process for preparing the same.

More specifically, this invention provides a cDNA clone for human hepatocyte inducible nitric oxide synthase and a process for preparing the same. This process includes inducing nitric oxide synthase in human hepatocytes, identifying human hepatocyte nitric oxide synthase messenger RNA, isolating the human hepatocyte nitric oxide synthase messenger RNA, collecting the human hepatocyte nitric oxide synthase messenger RNA, separating human hepatocyte poly A messenger RNA from the human hepatocyte nitric oxide synthase messenger RNA, constructing a cDNA library for human hepatocyte nitric oxide synthase,

screening this cDNA library for human hepatocyte inducible nitric oxide synthase cDNA clones, and converting the human hepatocyte inducible nitric oxide synthase cDNA clones to a plasmid vector for obtaining a substantially full length cDNA clone encoding human hepatocyte inducible nitric oxide synthase. This process further includes sequencing this cDNA, expressing the human hepatocyte inducible nitric oxide synthase cDNA protein in an expression system, and purifying the human hepatocyte inducible nitric oxide synthase cDNA protein.

It is an object of the present invention to provide for the molecular cloning and characterization of an inducible nitric oxide synthase in human tissues.

It is an object of the present invention to provide for the molecular cloning and characterization of an inducible nitric oxide synthase in human hepatocytes.

It is an object of the present invention to isolate a cDNA clone for human tissue inducible nitric oxide synthase.

It is an object of the present invention to isolate a cDNA clone for human hepatocyte inducible nitric oxide synthase.

It is an object of the present invention to provide a process for expressing and purifying human tissue inducible nitric oxide synthase enzyme.

It is an object of the present invention to provide a process for expressing and purifying human hepatocyte inducible nitric oxide synthase enzyme.

It is an object of this invention to provide for the regulation of gene expression for the human hepatocyte inducible nitric oxide synthase enzyme.

It is an object of this invention to provide for a protein including a human inducible nitric oxide synthase substantially free of other human proteins.

These and other objects of the invention will be more fully understood from the following description of the invention, the figures, the sequence listing and the claims appended hereto.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "patient" includes members of the animal kingdom including but not limited to human beings.

Nitric oxide is a biologic mediator derived from amino acid L-arginine. Nitric oxide synthase (NOS) acts upon L-arginine to oxidize one of the guanidino nitrogens to nitric oxide while citrulline is formed from the remainder of the L-arginine molecule. While it is understood by those skilled in the art that nitric oxide has both normal physiologic intracellular and extracellular regulatory functions, excessive production of nitric oxide is detrimental. It will be appreciated by those skilled in the art that there are no other readily available sources of human tissue inducible nitric oxide synthase. The present invention provides a cDNA clone for human tissue inducible nitric oxide synthase and a process for preparing the same. Therefore, the cloning and expression of a human tissue nitric oxide synthase cDNA of the present invention provides for a source of the enzyme for developing selective inhibitors of nitric oxide synthase.

The cloning and expression of a human tissue nitric oxide synthase cDNA of the present invention provides for a source of the enzyme in a sufficiently high concentration for providing a therapeutic purpose.

In one embodiment of this invention, a process for preparing a cDNA clone coding for a human tissue inducible nitric oxide synthase is provided. This process includes inducing the human tissue nitric oxide synthase in vitro, identifying the human tissue nitric oxide synthase messenger RNA (mRNA) by employing a cDNA probe capable of hybridizing with the human tissue inducible nitric oxide synthase mRNA, isolating the human tissue nitric oxide synthase mRNA, collecting the human tissue nitric oxide synthase mRNA, separating human tissue poly A mRNA from the human tissue nitric oxide synthase mRNA, constructing a human tissue inducible nitric oxide synthase cDNA library from the human tissue poly A mRNA using a reverse transcriptase enzyme and inserting a strand of the cDNA into a

phage vector, screening the cDNA library for human tissue inducible nitric oxide synthase clones including incubating the phage vector containing the cDNA with a bacteria for forming at least one positive plaque containing the cDNA clone for human tissue inducible nitric oxide synthase, rescuing the cDNA clone from the phage vector by employing a helper phage, and converting the rescued cDNA clone to a plasmid vector for obtaining a substantially full length cDNA clone encoding human tissue inducible nitric oxide synthase.

In another embodiment of this invention, this process, as hereinbefore described, further includes excising cDNA inserts for human tissue inducible nitric oxide synthase from the plasmid vector. This process also includes confirming the cDNA inserts by employing a dideoxynucleotide DNA sequencing. Further, this process includes confirming the cDNA inserts by employing Southern blot hybridization.

In another embodiment of this invention, the process, as hereinbefore described, includes expressing the human tissue inducible nitric oxide synthase cDNA protein in an expression system, such as for example, a bacterial expression system or a mammalian expression system.

It will be appreciated by those skilled in the art that the cloned human inducible nitric oxide synthase cDNA obtained through the methods described herein may be recombinantly expressed by molecular cloning into an expression vector containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant inducible nitric oxide synthase. Techniques for such manipulations are fully described in Maniatis, et al., infra, and are well known in the art.

Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned copies of genes and the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic genes in a variety of

hosts such as for example bacteria, bluegreen algae, plant cells, insect cells and animal cells.

Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast or bacteria-animal cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. A variety of mammalian expression vectors may be used to express recombinant inducible nitric oxide synthase in mammalian cells.

Commercially available bacterial expression vectors which may be suitable for recombinant inducible nitric oxide synthase expression, include but are not limited to, pKC30 (ATCC 37286), pPLa2311 (ATCC 31694), pBR322 (ATCC 31344 and 37017), ptac12 (ATCC 37138), lambda gt11 (ATCC 37194), pAS1 (ATCC39262), pLC24, pSB226, SV40 and pKK 223-3.

Commercially available mammalian expression vectors which may be suitable for recombinant inducible nitric oxide synthase expression, include but are not limited to, pBC12B1 (ATCC 67617), pMClneo (Stratagene), pXTI (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and lambda ZD35 (ATCC 37565).

DNA encoding inducible nitric oxide synthase may also be cloned into an expression vector for expression in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to bacteria, yeast, mammalian cells including but not limited to cell lines of human, bovine, porcine, monkey and rodent origin, and insect

cells including but not limited to drosophila derived cell lines. Cell lines derived from mammalian species which may be suitable and which are commercially available, include but are not limited to, CV-1 (ATCC CCL70), COS-1 (ATCC CRL1650), COS-7 (ATCC CRL1651), CHO-K1 (ATCC CCL61), 3T3 (ATCC CCL92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL2), C1271 (ATCC CRL1616), BS-C-1 (ATCC CCL26) and MRC-5 (ATCC CCL171). The bacterial cell most used for expression of recombinant protein is Escherichia coli. There are various strains of E. coli available and are well known in the art.

The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, protoplast fusion, and electroporation.

In a preferred embodiment of this invention, the process, as hereinbefore described, includes expressing the human tissue inducible nitric oxide synthase protein in a baculovirus expression system.

Another embodiment of this invention provides for a process, as hereinbefore described, including purifying the human tissue inducible nitric oxide synthase protein.

In a preferred embodiment of this invention, the process, as hereinbefore described, includes employing as the human tissue inducible nitric oxide synthase a human hepatocyte inducible nitric oxide synthase. This process further includes employing as the human tissue inducible nitric oxide synthase protein a human hepatocyte inducible nitric oxide synthase protein.

In another embodiment of this invention, a process is provided, as hereinbefore described, including inducing the human tissue nitric oxide synthase in vitro by stimulating a human tissue in vitro with at least one of the following (1) at least one cytokine, such as for example a cytokine selected from the group consisting of tissue necrosis factor (TNF), interleukin-1 (IL-1), and interferon-gamma (IFN-g), (2) at least

one bacterial endotoxin including, such as for example, a bacterial lipopolysaccharide (LPS) and (3) combinations thereof.

A further preferred embodiment of this invention provides a process, as hereinbefore described, that includes constructing a human tissue inducible nitric oxide synthase cDNA library from the human tissue poly A mRNA using a reverse transcriptase enzyme and inserting a cDNA strand having a length of about at least 1,000 base pairs into the phage vector. In yet another preferred embodiment, a process is provided, as hereinbefore described, that includes employing lambda Zap II as the phage vector.

In another embodiment of this invention, a process is provided, as hereinbefore described, including screening the cDNA library including incubating the phage vector for about 6 to 24 hours with a bacteria at a temperature from about 34 to 40 degrees centigrade for effectuating phage lysis of the bacteria. This process further includes rescuing the cDNA clone from the phage vector by employing a helper phage such as for example ExAssist helper phage (Stratagene, La Jolla, CA).

In a preferred embodiment of this invention, a process, as hereinbefore described, is provided including converting the rescued cDNA clone to the plasmid vector for obtaining a substantially full length cDNA clone encoding the human tissue inducible nitric oxide synthase wherein the plasmid vector includes pBluescript (Stratagene, La Jolla, CA).

In another preferred embodiment of this invention, a process as hereinbefore described is provided including employing as the human tissue inducible nitric oxide synthase a human hepatocyte inducible nitric oxide synthase.

Another embodiment of this invention provides for a process for producing human hepatocyte inducible nitric oxide synthase protein comprising providing a replicatable DNA expression vector capable of expressing a DNA sequence encoding human hepatocyte inducible nitric oxide synthase in a suitable host, transforming the host for obtaining a recombinant host, and maintaining the recombinant host under conditions permitting

expression of the DNA sequence to provide human hepatocyte inducible nitric oxide synthase.

Another embodiment of this invention provides a human tissue inducible nitric oxide synthase cDNA clone. A preferred embodiment of this invention provides a human hepatocyte inducible nitric oxide synthase cDNA clone. The human hepatocyte inducible nitric oxide synthase cDNA clone of this invention has a cDNA (SEQ ID NO:1) coding for the amino acid sequence (SEQ ID NOS: 1 and 2), shown in Figures 1A-G. Figures 1A-G show the cDNA sense sequence (top line of each horizontal row) and the deduced amino acid sequence of amino acids 1-1153 (bottom line of each horizontal row) for the cDNA clone for the human hepatocyte inducible nitric oxide synthase of this invention. Figures 1A-G show that the cDNA sequence for the human hepatocyte inducible nitric oxide synthase of this invention is 4,145 nucleotide bases long with the start codon beginning at base number 207 and the stop codon ending at base number 3668. The cDNA double strand sequence was determined using the Sanger dideoxynucleotide sequence technique well known by those skilled in the art on a Genesis 2000 sequencing system (USB, Cleveland, Ohio). Proc. Natl. Acad. Sci. USA, Vol 74, p. 5463 (1977).

Another embodiment of this invention provides a human tissue inducible nitric oxide synthase recombinant protein expressed from a human tissue inducible nitric oxide synthase cDNA clone. In a preferred embodiment, a human hepatocyte inducible nitric oxide synthase recombinant protein expressed from a human hepatocyte inducible nitric oxide synthase cDNA clone is provided.

Another embodiment of this invention provides for a protein comprising a human inducible nitric oxide synthase substantially free of other human proteins.

Another embodiment of this invention provides for an isolated DNA sequence encoding human inducible nitric oxide synthase consisting essentially of an initiation codon positioned upstream and adjacent to an open reading frame

consisting essentially of a DNA sequence encoding human inducible nitric oxide synthase.

A further embodiment of this invention provides for an isolated DNA sequence encoding human inducible nitric oxide synthase consisting essentially of an initiation codon positioned upstream and adjacent to an open reading frame consisting essentially of a DNA sequence encoding human inducible nitric oxide synthase protein. The human inducible nitric oxide synthase protein begins at the initiation codon and terminates at a stop codon.

In yet another embodiment of this invention a recombinant plasmid is provided containing a recombinant plasmid pHINOS having a deposit accession number ATCC 75358 deposited with the American Type Culture Collection. A further embodiment of this invention provides for bacteria transformed by the recombinant plasmid pHINOS.

In another embodiment of this invention a microorganism is provided containing a HINOS cDNA plasmid transformed in E. coli SOLR bacteria having a deposit accession number ATCC 69126 deposited with the American Type Culture Collection.

## EXAMPLE 1

### INDUCING HUMAN HEPATOCYTE INDUCIBLE NITRIC OXIDE SYNTHASE

mRNA is weakly induced following stimulation with cytokine signals such as for example tumor necrosis factor (TNF), interleukin-1 (IL-1) or interleukin-gamma (IFN-g). Cytokine signals synergize to further up-regulate mRNA levels and nitric oxide synthase activity. Maximum induction was achieved with a combination of TNF, IL-1, IFN-g and bacterial lipopolysaccharide (LPS). FASEB Journal, Vol. 6, supra, and J. Exp. Med., Vol. 176, supra.

EXAMPLE 2

IDENTIFYING AND ISOLATING HUMAN HEPATOCYTE NITRIC OXIDE SYNTHASE mRNA

A cDNA probe capable of hybridizing with human hepatocyte inducible nitric oxide synthase mRNA was used for identifying and isolating the mRNA for human hepatocyte inducible nitric oxide synthase. The time-point of peak mRNA levels following cytokine and LPS [hereinafter cytokine mixture (CM)] stimulation was then determined.

Total RNA was extracted about 2-48 hours following CM-stimulation of cultured human hepatocytes using the RNAzol B modified method of Chomczynski and Sacchi. Anal Biochem., Vol 162; pp. 156-159 (1987). Northern blot analysis was performed on 20 microgram (ug) aliquots of total RNA using a murine macrophage cDNA probe, representing excision fragment produced by Not I restriction enzyme [Proc. Natl. Acad. Sci. USA., Vol 89, pp. 6711-6715 (1992) GenBank Accession No. M92649] and cross-species hybridization. The human hepatocyte nitric oxide synthase mRNA was identified as a single band at about 4.5 kb (kilobase) with maximal mRNA levels seen about 8 hours after stimulation.

Figure 2 shows the presence of the 4.5 kb message for human hepatocyte inducible nitric oxide synthase. Human hepatocytes (HC) that were freshly isolated were placed in cell culture and exposed to a combination of human recombinant tumor necrosis factor (500 units/milliliter), human recombinant interleukin-1 (5 units/milliliter), human recombinant interferon-gamma (100 units/milliliter), and lipopolysaccharide (10 micrograms/milliliter). Figure 2 shows that at the indicated time points (2 hours, 4 hours, 6 hours and 8 hours) total RNA was isolated and that 20 micrograms per sample was subjected to Northern Blot analysis. A 2.7 Kb fragment of cDNA to murine macrophage inducible nitric oxide synthase was used to hybridize with the mRNA for human hepatocyte inducible nitric oxide synthase. Figure 2 demonstrates that the 4.5 Kb message peaked at about 8 hours following stimulation. Figure 2 shows that no

mRNA signal was detected in control (unstimulated) hepatocytes. Figure 3 shows the expression of the 4.5 Kb mRNA for human hepatocyte inducible nitric oxide synthase at about 8 hours after exposure to the above mentioned signals for hepatocytes isolated from three separate individuals [patient (Pt.) 1, 2, and 3]. Figure 3 demonstrates that no signal was detected in control (unstimulated) hepatocytes.

Because the 8 hour time point yielded maximal mRNA levels, samples of RNA were isolated from two human livers about 8 hours following CM-stimulation in vitro and were pooled to obtain sufficient quantity for the cDNA library construction. The cDNA synthesis requires about from 10 to 20 micrograms of poly A mRNA rather than total RNA. To obtain purified poly A mRNA, poly A mRNA was separated from total RNA by elution through an oligo-dT cellulose column. The purity of the mRNA was assessed by repeat Northern blot analysis which included subjecting 0.5 micrograms of poly A RNA from each of the two human livers to Northern Blot analysis using the 2.7 Kb cDNA from murine macrophage inducible nitric oxide synthase. Figure 4 shows strong nitric oxide synthase mRNA bands from 2 different patients without evidence of degraded poly A RNA.

Figure 4 shows that the murine macrophage inducible nitric oxide synthase cross hybridizes with the human hepatocyte inducible nitric oxide synthase poly A RNA and effectively identifies the mRNA for human hepatocyte inducible nitric oxide synthase. These samples of poly A RNA were used to construct the cDNA library for isolation of the cDNA clone for the human hepatocyte inducible nitric oxide synthase.

## EXAMPLE 3

### CONSTRUCTING A HUMAN HEPATOCYTE INDUCIBLE NITRIC OXIDE SYNTHASE cDNA LIBRARY

Using about 20 micrograms of poly A RNA enriched for hepatocyte nitric oxide synthase mRNA by CM-stimulation, a cDNA library was constructed by Stratagene, La Jolla, CA. The first strand cDNA was synthesized from the human hepatocyte poly A RNA

using reverse transcriptase enzyme with random and oligo-dT primers. After size exclusion for a minimum of about 1000 nucleotide base pair length, the cDNA's were inserted into a lambda Zap II phage vector (Stratagene, La Jolla, CA) and was titered.

## EXAMPLE 4

## SCREENING THE cDNA LIBRARY FOR HUMAN HEPATOCYTE INDUCIBLE NITRIC OXIDE SYNTHASE cDNA CLONES

To screen the cDNA library, 1 x $10^6$ phage were incubated with bacteria (E. coli Sure strain) at about 34 to 40 degrees centigrade for about 15 to 30 minutes. This mixture was added to molten agarose and poured onto 20 x 20 centimeter agar plates at a density of about 2 x $10^5$ plaques/plate (Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982). The plates were incubated from about 34 to 40 degrees centigrade overnight from about 6 to 24 hours to allow for phage lysis of bacteria. The plaques were then transferred to nylon filters and positive clones were identified by filter hybridization with $^{32}$P-labeled murine macrophage nitric oxide synthase cDNA probe. Positive clones were cored from the agar plates after localization by autoradiograph alignment. This procedure was repeated about 3 times until individual clones were isolated. The positive clones were rescued from the lambda Zap II phage vector using a helper phage ExAssist (Stratagene, La Jolla, CA), and then converted to the plasmid vector, pBluescript (Stratagene, La Jolla, CA). The cDNA inserts for human hepatocyte inducible nitric oxide synthase were excised from the Bluescript plasmid cloning sites by restriction analysis with EcoRI enzyme and then sized by gel electrophoresis. The cDNA insert identities were confirmed by DNA sequencing and by Southern blot hybridization with the murine macrophage cDNA clone. In addition, repeat Northern blot analysis was performed on cytokine-stimulated human hepatocytes in culture using the human nitric oxide synthase cDNA clone of this invention as probe. Figure 5 shows

a time course for mRNA expression for human hepatocyte inducible nitric oxide synthase. This RNA is from an individual patient different from the patients listed in Figures 2 and 3. The cells of the patient in Figure 5 were exposed to the same agents as described for Figure 2. Figure 5 shows the human nitric oxide synthase cDNA identifying the same mRNA signal as the macrophage probe, thus, further confirming its identify. It is important to note that the isolated cDNA clone coding for human inducible nitric oxide synthase of this invention was used to hybridize with the mRNA, thus, confirming the capacity of the cDNA clone of this invention to identify the human hepatocyte inducible nitric oxide synthase mRNA.

## EXAMPLE 5

## cDNA SEQUENCING

The plasmid vector pBluescript contains universal primer regions which were used to facilitate double-stranded DNA sequencing. Positive clones were sequenced by using the dideoxynucleotide technique of Sanger, supra, with the Genesis 2000 sequencing system (USB, Cleveland, Ohio). Sequence analysis was done using Genbank DNA sequencing software programs available through the Pittsburgh Supercomputing Center (Billiar TR., Pittsburgh Supercomputing Center, Pittsburgh, PA).

## EXAMPLE 6

## EXPRESSING HUMAN HEPATOCYTE INDUCIBLE NITRIC OXIDE SYNTHASE

Verification of the full length cDNA identify was accomplished by expressing the recombinant human hepatocyte inducible nitric oxide synthase protein. The human hepatocyte inducible nitric oxide synthase clone was ligated into the pCIS expression vector (Genentech, CA) which utilizes a CMV promoter. Next the expression vector was transfected into human embryonic kidney 293 cells (ATCC, Maryland). Nitric oxide synthase activity was assessed by measuring the conversion of [$^3$H] arginine to [$^3$H] citrulline. It will be appreciated by those

skilled in the art that this expression system was successfully used for expression of the cloned rat brain constitutive nitric oxide synthase, and there was negligible nitric oxide synthase activity in the unstimulated 293 kidney cells [Bredt et al., Nature, Vol 351, p. 714 (1991)]. After the identity of the human hepatocyte inducible nitric oxide synthase clone of this invention was verified as hereinbefore described, the cDNA was expressed in a baculovirus expression system (Invitrogen, San Diego, CA) which allowed for large scale enzyme production. Texas Agriculture Experiment Station Bulletin, No. 1555 (1988). More specifically, the human hepatocyte nitric oxide synthase cDNA was inserted into the baculovirus transfer vector and then co-transfected with wild type viral DNA into Sf9 insect cells (ATCC, Maryland). Recombinant viral plaques were isolated to allow for protein over-expression.

## EXAMPLE 7

### PURIFYING THE HUMAN HEPATOCYTE INDUCIBLE NITRIC OXIDE SYNTHASE PROTEIN

The resultant human hepatocyte inducible nitric synthase protein was purified using a two step procedure. First, the protein was passed through an anion-exchange column of DEAE cellulose. This was followed by affinity chromatography with 2', 5'-ADP Sepharose. [Evans et al., Proc. Natl. Acad. Sci. USA, Vol. 89, pp. 5361-5365 (1992)] Purity was assessed by SDS-polyacrylamide gel electrophoresis. Activity was quantitated after each step by measuring the ability of the enzyme to generate $NO_2$- and $NO_3$- from L-arginine. $NO_2$- and $NO_3$- was measured using an automated colorimetric reaction based on the Greiss reaction [Green et al., Anal. Biochem., Vol. 126, p. 131 (1982)].

Whereas, particular embodiments of this invention have been described above for purposes of illustration, it will be evident to those persons skilled in the art that numerous variations of the details of the present invention may be made without departing from the invention as defined in the appended claims that follow the SEQUENCE LISTING.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Billiar, Timothy R.
Nussler, Andreas K.
Geller, David A.
Simmons, Richard L.

(ii) TITLE OF INVENTION: cDNA Clone for Human Inducible Nitric Oxide Synthase And Process for Preparing Same

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Arnold B. Silverman
Eckert Seamans Cherin & Mellott
(B) STREET: 600 Grant Street, 42nd Floor
(C) CITY: Pittsburgh
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 15219

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/981,344
(B) FILING DATE: 25-NOV-1992
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Silverman, Arnold B.
(B) REGISTRATION NUMBER: 22,614
(C) REFERENCE/DOCKET NUMBER: 116972

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (412) 566-6000
(B) TELEFAX: (412) 566-6099

(C) TELEX: 866172

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4145 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(A) Human Hepatocyte Inducible Nitric Oxide Synthase cDNA Clone

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: Induced Human Hepatocyte RNA

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Lambda Zap II cDNA
(B) CLONE: pHINOS

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: unknown
(B) MAP POSITION: unknown
(C) UNITS: unknown

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 207..3668
(C) IDENTIFICATION METHOD: Experiment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTGCTTTAAA ATCTCTCGGC CACCTTTGAT GAGGGGACTG GGCAGTTCTA GACAGTCCCG 60

AAGTTCTCAA GGCACAGGTC TCTTCCTGGT TTGACTGTCC TTACCCCGGG GAGGCAGTGC 120

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AGCCAGCTGC AAGCCCCACA GTGAAGAACA TCTGAGCTCA AATCCAGATA AGTGACATAA      180

GTGACCTGCT TTGTAAAGCC ATAGAG ATG GCC TGT CCT TGG AAA TTT CTG TTC       233
                             Met Ala Cys Pro Trp Lys Phe Leu Phe
                               1               5

AAG ACC AAA TTC CAC CAG TAT GCA ATG AAT GGG GAA AAA GAC ATC AAC        281
Lys Thr Lys Phe His Gln Tyr Ala Met Asn Gly Glu Lys Asp Ile Asn
 10                  15                  20                  25

AAC AAT GTG GAG AAA GCC CCC TGT GCC ACC TCC AGT CCA GTG ACA CAG        329
Asn Asn Val Glu Lys Ala Pro Cys Ala Thr Ser Ser Pro Val Thr Gln
                 30                  35                  40

GAT GAC CTT CAG TAT CAC AAC CTC AGC AAG CAG CAG AAT GAG TCC CCG        377
Asp Asp Leu Gln Tyr His Asn Leu Ser Lys Gln Gln Asn Glu Ser Pro
             45                  50                  55

CAG CCC CTC GTG GAG ACG GGA AAG AAG TCT CCA GAA TCT CTG GTC AAG        425
Gln Pro Leu Val Glu Thr Gly Lys Lys Ser Pro Glu Ser Leu Val Lys
         60                  65                  70

CTG GAT GCA ACC CCA TTG TCC TCC CCA CGG CAT GTG AGG ATC AAA AAC        473
Leu Asp Ala Thr Pro Leu Ser Ser Pro Arg His Val Arg Ile Lys Asn
     75                  80                  85

TGG GGC AGC GGG ATG ACT TTC CAA GAC ACA CTT CAC CAT AAG GCC AAA        521
Trp Gly Ser Gly Met Thr Phe Gln Asp Thr Leu His His Lys Ala Lys
 90                  95                  100                 105

GGG ATT TTA ACT TGC AGG TCC AAA TCT TGC CTG GGG TCC ATT ATG ACT        569
Gly Ile Leu Thr Cys Arg Ser Lys Ser Cys Leu Gly Ser Ile Met Thr
                110                 115                 120

CCC AAA AGT TTG ACC AGA GGA CCC AGG GAC AAG CCT ACC CCT CCA GAT        617
Pro Lys Ser Leu Thr Arg Gly Pro Arg Asp Lys Pro Thr Pro Pro Asp
            125                 130                 135

GAG CTT CTA CCT CAA GCT ATC GAA TTT GTC AAC CAA TAT TAC GGC TCC        665
Glu Leu Leu Pro Gln Ala Ile Glu Phe Val Asn Gln Tyr Tyr Gly Ser
        140                 145                 150

TTC AAA GAG GCA AAA ATA GAG GAA CAT CTG GCC AGG GTG GAA GCG GTA        713
Phe Lys Glu Ala Lys Ile Glu Glu His Leu Ala Arg Val Glu Ala Val
    155                 160                 165

ACA AAG GAG ATA GAA ACA ACA GGA ACC TAC CAA CTG ACG GGA GAT GAG        761
Thr Lys Glu Ile Glu Thr Thr Gly Thr Tyr Gln Leu Thr Gly Asp Glu
170                 175                 180                 185

CTC ATC TTC GCC ACC AAG CAG GCC TGG CGC AAT GCC CCA CGC TGC ATT        809
Leu Ile Phe Ala Thr Lys Gln Ala Trp Arg Asn Ala Pro Arg Cys Ile
                190                 195                 200

GGG AGG ATC CAG TGG TCC AAC CTG CAG GTC TTC GAT GCC CGC AGC TGT        857
Gly Arg Ile Gln Trp Ser Asn Leu Gln Val Phe Asp Ala Arg Ser Cys
            205                 210                 215
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TCC ACT GCC CGG GAA ATG TTT GAA CAC ATC TGC AGA CAC GTG CGT TAC        905
Ser Thr Ala Arg Glu Met Phe Glu His Ile Cys Arg His Val Arg Tyr
        220                 225                 230

TCC ACC AAC AAT GGC AAC ATC AGG TCG GCC ATC ACC GTG TTC CCC CAG        953
Ser Thr Asn Asn Gly Asn Ile Arg Ser Ala Ile Thr Val Phe Pro Gln
    235                 240                 245

CGG AGT GAT GGC AAG CAC GAC TTC CGG GTG TGG AAT GCT CAG CTC ATC       1001
Arg Ser Asp Gly Lys His Asp Phe Arg Val Trp Asn Ala Gln Leu Ile
250                 255                 260                 265

CGC TAT GCT GGC TAC CAG ATG CCA GAT GGC AGC ATC AGA GGG GAC CCT       1049
Arg Tyr Ala Gly Tyr Gln Met Pro Asp Gly Ser Ile Arg Gly Asp Pro
                270                 275                 280

GCC AAC GTG GAA TTC ACT CAG CTG TGC ATC GAC CTG GGC TGG AAG CCC       1097
Ala Asn Val Glu Phe Thr Gln Leu Cys Ile Asp Leu Gly Trp Lys Pro
            285                 290                 295

AAG TAC GGC CGC TTC GAT GTG GTC CCC CTG GTC CTG CAG GCC AAT GGC       1145
Lys Tyr Gly Arg Phe Asp Val Val Pro Leu Val Leu Gln Ala Asn Gly
        300                 305                 310

CGT GAC CCT GAG CTC TTC GAA ATC CCA CCT GAC CTT GTG CTT GAG GTG       1193
Arg Asp Pro Glu Leu Phe Glu Ile Pro Pro Asp Leu Val Leu Glu Val
    315                 320                 325

GCC ATG GAA CAT CCC AAA TAC GAG TGG TTT CGG GAA CTG GAG CTA AAG       1241
Ala Met Glu His Pro Lys Tyr Glu Trp Phe Arg Glu Leu Glu Leu Lys
330                 335                 340                 345

TGG TAC GCC CTG CCT GCA GTG GCC AAC ATG CTG CTT GAG GTG GGC GGC       1289
Trp Tyr Ala Leu Pro Ala Val Ala Asn Met Leu Leu Glu Val Gly Gly
                350                 355                 360

CTG GAG TTC CCA GGG TGC CCC TTC AAT GGC TGG TAC ATG GGC ACA GAG       1337
Leu Glu Phe Pro Gly Cys Pro Phe Asn Gly Trp Tyr Met Gly Thr Glu
            365                 370                 375

ATC GGA GTC CGG GAC TTC TGT GAC GTC CAG CGC TAC AAC ATC CTG GAG       1385
Ile Gly Val Arg Asp Phe Cys Asp Val Gln Arg Tyr Asn Ile Leu Glu
        380                 385                 390

GAA GTG GGC AGG AGA ATG GGC CTG GAA ACG CAC AAG CTG GCC TCG CTC       1433
Glu Val Gly Arg Arg Met Gly Leu Glu Thr His Lys Leu Ala Ser Leu
    395                 400                 405

TGG AAA GAC CAG GCT GTC GTT GAG ATC AAC ATT GCT GTG ATC CAT AGT       1481
Trp Lys Asp Gln Ala Val Val Glu Ile Asn Ile Ala Val Ile His Ser
410                 415                 420                 425

TTT CAG AAG CAG AAT GTG ACC ATC ATG GAC CAC CAC TCG GCT GCA GAA       1529
Phe Gln Lys Gln Asn Val Thr Ile Met Asp His His Ser Ala Ala Glu
                430                 435                 440
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TCC TTC ATG AAG TAC ATG CAG AAT GAA TAC CGG TCC CGT GGG GGC TGC      1577
Ser Phe Met Lys Tyr Met Gln Asn Glu Tyr Arg Ser Arg Gly Gly Cys
            445                 450                 455

CCG GCA GAC TGG ATT TGG CTG GTC CCT CCC ATG TCT GGG AGC ATC ACC      1625
Pro Ala Asp Trp Ile Trp Leu Val Pro Pro Met Ser Gly Ser Ile Thr
        460                 465                 470

CCC GTG TTT CAC CAG GAG ATG CTG AAC TAC GTC CTG TCC CCT TTC TAC      1673
Pro Val Phe His Gln Glu Met Leu Asn Tyr Val Leu Ser Pro Phe Tyr
    475                 480                 485

TAC TAT CAG GTA GAG GCC TGG AAA ACC CAT GTC TGG CAG GAC GAG AAG      1721
Tyr Tyr Gln Val Glu Ala Trp Lys Thr His Val Trp Gln Asp Glu Lys
490                 495                 500                 505

CGG AGA CCC AAG AGA AGA GAG ATT CCA TTG AAA GTC TTG GTC AAA GCT      1769
Arg Arg Pro Lys Arg Arg Glu Ile Pro Leu Lys Val Leu Val Lys Ala
                510                 515                 520

GTG CTC TTT GCC TGT ATG CTG ATG CGC AAG ACA ATG GCG TCC CGA GTC      1817
Val Leu Phe Ala Cys Met Leu Met Arg Lys Thr Met Ala Ser Arg Val
            525                 530                 535

AGA GTC ACC ATC CTC TTT GCG ACA GAG ACA GGA AAA TCA GAG GCG CTG      1865
Arg Val Thr Ile Leu Phe Ala Thr Glu Thr Gly Lys Ser Glu Ala Leu
        540                 545                 550

GCC TGG GAC CTG GGG GCC TTA TTC AGC TGT GCC TTC AAC CCC AAG GTT      1913
Ala Trp Asp Leu Gly Ala Leu Phe Ser Cys Ala Phe Asn Pro Lys Val
    555                 560                 565

GTC TGC ATG GAT AAG TAC AGG CTG AGC TGC CTG GAG GAG GAA CGG CTG      1961
Val Cys Met Asp Lys Tyr Arg Leu Ser Cys Leu Glu Glu Glu Arg Leu
570                 575                 580                 585

CTG TTG GTG GTG ACC AGT ACG TTT GGC AAT GGA GAC TGC CCT GGC AAT      2009
Leu Leu Val Val Thr Ser Thr Phe Gly Asn Gly Asp Cys Pro Gly Asn
                590                 595                 600

GGA GAG AAA CTG AAG AAA TCG CTC TTC ATG CTG AAA GAG CTC AAC AAC      2057
Gly Glu Lys Leu Lys Lys Ser Leu Phe Met Leu Lys Glu Leu Asn Asn
            605                 610                 615

AAA TTC AGG TAC GCT GTG TTT GGC CTC GGC TCC AGC ATG TAC CCT CGG      2105
Lys Phe Arg Tyr Ala Val Phe Gly Leu Gly Ser Ser Met Tyr Pro Arg
        620                 625                 630

TTC TGC GCC TTT GCT CAT GAC ATT GAT CAG AAG CTG TCC CAC CTG GGG      2153
Phe Cys Ala Phe Ala His Asp Ile Asp Gln Lys Leu Ser His Leu Gly
    635                 640                 645

GCC TCT CAG CTC ACC CCG ATG GGA GAA GGG GAT GAG CTC AGT GGG CAG      2201
Ala Ser Gln Leu Thr Pro Met Gly Glu Gly Asp Glu Leu Ser Gly Gln
650                 655                 660                 665
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GAG GAC GCC TTC CGC AGC TGG GCC GTG CAA ACC TTC AAG GCA GCC TGT      2249
Glu Asp Ala Phe Arg Ser Trp Ala Val Gln Thr Phe Lys Ala Ala Cys
                670                 675                 680

GAG ACG TTT GAT GTC CGA GGC AAA CAG CAC ATT CAG ATC CCC AAG CTC      2297
Glu Thr Phe Asp Val Arg Gly Lys Gln His Ile Gln Ile Pro Lys Leu
            685                 690                 695

TAC ACC TCC AAT GTG ACC TGG GAC CCG CAC CAC TAC AGG CTC GTG CAG      2345
Tyr Thr Ser Asn Val Thr Trp Asp Pro His His Tyr Arg Leu Val Gln
        700                 705                 710

GAC TCA CAG CCT TTG GAC CTC AGC AAA GCC CTC AGC AGC ATG CAT GCC      2393
Asp Ser Gln Pro Leu Asp Leu Ser Lys Ala Leu Ser Ser Met His Ala
    715                 720                 725

AAG AAC GTG TTC ACC ATG AGG CTC AAA TCT CGG CAG AAT CTA CAA AGT      2441
Lys Asn Val Phe Thr Met Arg Leu Lys Ser Arg Gln Asn Leu Gln Ser
730                 735                 740                 745

CCG ACA TCC AGC CGT GCC ACC ATC CTG GTG GAA CTC TCC TGT GAG GAT      2489
Pro Thr Ser Ser Arg Ala Thr Ile Leu Val Glu Leu Ser Cys Glu Asp
                750                 755                 760

GGC CAA GGC CTG AAC TAC CTG CCG GGG GAG CAC CTT GGG GTT TGC CCA      2537
Gly Gln Gly Leu Asn Tyr Leu Pro Gly Glu His Leu Gly Val Cys Pro
            765                 770                 775

GGC AAC CAG CCG GCC CTG GTC CAA GGC ATC CTG GAG CGA GTG GTG GAT      2585
Gly Asn Gln Pro Ala Leu Val Gln Gly Ile Leu Glu Arg Val Val Asp
        780                 785                 790

GGC CCC ACA CCC CAC CAG ACA GTG CGC CTG GAG GAC CTG GAT GAG AGT      2633
Gly Pro Thr Pro His Gln Thr Val Arg Leu Glu Asp Leu Asp Glu Ser
    795                 800                 805

GGC AGC TAC TGG GTC AGT GAC AAG AGG CTG CCC CCC TGC TCA CTC AGC      2681
Gly Ser Tyr Trp Val Ser Asp Lys Arg Leu Pro Pro Cys Ser Leu Ser
810                 815                 820                 825

CAG GCC CTC ACC TAC TCC CCG GAC ATC ACC ACA CCC CCA ACC CAG CTG      2729
Gln Ala Leu Thr Tyr Ser Pro Asp Ile Thr Thr Pro Pro Thr Gln Leu
                830                 835                 840

CTG CTC CAA AAG CTG GCC CAG GTG GCC ACA GAA GAG CCT GAG AGA CAG      2777
Leu Leu Gln Lys Leu Ala Gln Val Ala Thr Glu Glu Pro Glu Arg Gln
            845                 850                 855

AGG CTG GAG GCC CTG TGC CAG CCC TCA GAG TAC AGC AAG TGG AAG TTC      2825
Arg Leu Glu Ala Leu Cys Gln Pro Ser Glu Tyr Ser Lys Trp Lys Phe
        860                 865                 870

ACC AAC AGC CCC ACA TTC CTG GAG GTG CTA GAG GAG TTC CCG TCC CTG      2873
Thr Asn Ser Pro Thr Phe Leu Glu Val Leu Glu Glu Phe Pro Ser Leu
    875                 880                 885
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CGG GTG TCT GCT GGC TTC CTG CTT TCC CAG CTC CCC ATT CTG AAG CCC      2921
Arg Val Ser Ala Gly Phe Leu Leu Ser Gln Leu Pro Ile Leu Lys Pro
890                 895                 900                 905

AGG TTC TAC TCC ATC AGC TCC TCC CGG GAT CAC ACG CCC ACG GAG ATC      2969
Arg Phe Tyr Ser Ile Ser Ser Ser Arg Asp His Thr Pro Thr Glu Ile
                910                 915                 920

CAC CTG ACT GTG GCC GTG GTC ACC TAC CAC ACC GGA GAT GGC CAG GGT      3017
His Leu Thr Val Ala Val Val Thr Tyr His Thr Gly Asp Gly Gln Gly
            925                 930                 935

CCC CTG CAC CAC GGT GTC TGC AGC ACA TGG CTC AAC AGC CTG AAG CCC      3065
Pro Leu His His Gly Val Cys Ser Thr Trp Leu Asn Ser Leu Lys Pro
        940                 945                 950

CAA GAC CCA GTG CCC TGC TTT GTG CGG AAT GCC AGC GCC TTC CAC CTC      3113
Gln Asp Pro Val Pro Cys Phe Val Arg Asn Ala Ser Ala Phe His Leu
    955                 960                 965

CCC GAG GAT CCC TCC CAT CCT TGC ATC CTC ATC GGG CCT GGC ACA GGC      3161
Pro Glu Asp Pro Ser His Pro Cys Ile Leu Ile Gly Pro Gly Thr Gly
970                 975                 980                 985

ATC GTG CCC TTC CGC AGT TTC TGG CAG CAA CGG CTC CAT GAC TCC CAG      3209
Ile Val Pro Phe Arg Ser Phe Trp Gln Gln Arg Leu His Asp Ser Gln
                990                 995                1000

CAC AAG GGA GTG CGG GGA GGC CGC ATG ACC TTG GTG TTT GGG TGC CGC      3257
His Lys Gly Val Arg Gly Gly Arg Met Thr Leu Val Phe Gly Cys Arg
            1005                1010                1015

CGC CCA GAT GAG GAC CAC ATC TAC CAG GAG GAG ATG CTG GAG ATG GCC      3305
Arg Pro Asp Glu Asp His Ile Tyr Gln Glu Glu Met Leu Glu Met Ala
        1020                1025                1030

CAG AAG GGG GTG CTG CAT GCG GTG CAC ACA GCC TAT TCC CGC CTG CCT      3353
Gln Lys Gly Val Leu His Ala Val His Thr Ala Tyr Ser Arg Leu Pro
    1035                1040                1045

GGC AAG CCC AAG GTC TAT GTT CAG GAC ATC CTG CGG CAG CAG CTG GCC      3401
Gly Lys Pro Lys Val Tyr Val Gln Asp Ile Leu Arg Gln Gln Leu Ala
1050                1055                1060                1065

AGC GAG GTG CTC CGT GTG CTC CAC AAG GAG CCA GGC CAC CTC TAT GTT      3449
Ser Glu Val Leu Arg Val Leu His Lys Glu Pro Gly His Leu Tyr Val
                1070                1075                1080

TGC GGG GAT GTG CGC ATG GCC CGG GAC GTG GCC CAC ACC CTG AAG CAG      3497
Cys Gly Asp Val Arg Met Ala Arg Asp Val Ala His Thr Leu Lys Gln
            1085                1090                1095

CTG GTG GCT GCC AAG CTG AAA TTG AAT GAG GAG CAG GTC GAG GAC TAT      3545
Leu Val Ala Ala Lys Leu Lys Leu Asn Glu Glu Gln Val Glu Asp Tyr
        1100                1105                1110
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TTC TTT CAG CTC AAG AGC CAG AAG CGC TAT CAC GAA GAT ATC TTC GGT      3593
Phe Phe Gln Leu Lys Ser Gln Lys Arg Tyr His Glu Asp Ile Phe Gly
    1115                1120                1125

GCT GTA TTT CCT TAC GAG GCG AAG AAG GAC AGG GTG GCG GTG CAG CCC      3641
Ala Val Phe Pro Tyr Glu Ala Lys Lys Asp Arg Val Ala Val Gln Pro
1130                1135                1140                1145

AGC AGC CTG GAG ATG TCA GCG CTC TGAGGGCCTA CAGGAGGGGT TAAAGCTGCC     3695
Ser Ser Leu Glu Met Ser Ala Leu
                1150

GGCACAGAAC TTAAGGATGG AGCCAGCTCT GCATTATCTG AGGTCACAGG GCCTGGGGAG    3755

ATGGAGGAAA GTGATATCCC CCAGCCTCAA GTCTTATTTC CTCAACGTTG CTCCCCATCA    3815

AGCCCTTTAC TTGACCTCCT AACAAGTAGC ACCCTGGATT GATCGGAGCC TCCTCTCTCA    3875

AACTGGGGCC TCCCTGGTCC CTTGGAGACA AAATCTTAAA TGCCAGGCCT GGCGAGTGGG    3935

TGAAAGATGG AACTTGCTGC TGAGTGCACC ACTTCAAGTG ACCACCAGGA GGTGCTATCG    3995

CACCACTGTG TATTTAACTG CCTTGTGTAC AGTTATTTAT GCCTCTGTAT TTAAAAAACT    4055

AACACCCAGT CTGTTCCCCA TGGCCACTTG GGTCTTCCCT GTATGATTCC TTGATGGAGA    4115

TATTTACATG AATTGCATTT TACTTTAATC                                     4145
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1153 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ala Cys Pro Trp Lys Phe Leu Phe Lys Thr Lys Phe His Gln Tyr
 1               5                  10                  15

Ala Met Asn Gly Glu Lys Asp Ile Asn Asn Asn Val Glu Lys Ala Pro
            20                  25                  30

Cys Ala Thr Ser Ser Pro Val Thr Gln Asp Asp Leu Gln Tyr His Asn
        35                  40                  45

Leu Ser Lys Gln Gln Asn Glu Ser Pro Gln Pro Leu Val Glu Thr Gly
    50                  55                  60
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Lys Lys Ser Pro Glu Ser Leu Val Lys Leu Asp Ala Thr Pro Leu Ser
 65                  70                  75                  80

Ser Pro Arg His Val Arg Ile Lys Asn Trp Gly Ser Gly Met Thr Phe
                 85                  90                  95

Gln Asp Thr Leu His His Lys Ala Lys Gly Ile Leu Thr Cys Arg Ser
            100                 105                 110

Lys Ser Cys Leu Gly Ser Ile Met Thr Pro Lys Ser Leu Thr Arg Gly
        115                 120                 125

Pro Arg Asp Lys Pro Thr Pro Pro Asp Glu Leu Leu Pro Gln Ala Ile
    130                 135                 140

Glu Phe Val Asn Gln Tyr Tyr Gly Ser Phe Lys Glu Ala Lys Ile Glu
145                 150                 155                 160

Glu His Leu Ala Arg Val Glu Ala Val Thr Lys Glu Ile Glu Thr Thr
                165                 170                 175

Gly Thr Tyr Gln Leu Thr Gly Asp Glu Leu Ile Phe Ala Thr Lys Gln
            180                 185                 190

Ala Trp Arg Asn Ala Pro Arg Cys Ile Gly Arg Ile Gln Trp Ser Asn
        195                 200                 205

Leu Gln Val Phe Asp Ala Arg Ser Cys Ser Thr Ala Arg Glu Met Phe
    210                 215                 220

Glu His Ile Cys Arg His Val Arg Tyr Ser Thr Asn Asn Gly Asn Ile
225                 230                 235                 240

Arg Ser Ala Ile Thr Val Phe Pro Gln Arg Ser Asp Gly Lys His Asp
                245                 250                 255

Phe Arg Val Trp Asn Ala Gln Leu Ile Arg Tyr Ala Gly Tyr Gln Met
            260                 265                 270

Pro Asp Gly Ser Ile Arg Gly Asp Pro Ala Asn Val Glu Phe Thr Gln
        275                 280                 285

Leu Cys Ile Asp Leu Gly Trp Lys Pro Lys Tyr Gly Arg Phe Asp Val
    290                 295                 300

Val Pro Leu Val Leu Gln Ala Asn Gly Arg Asp Pro Glu Leu Phe Glu
305                 310                 315                 320

Ile Pro Pro Asp Leu Val Leu Glu Val Ala Met Glu His Pro Lys Tyr
                325                 330                 335

Glu Trp Phe Arg Glu Leu Glu Leu Lys Trp Tyr Ala Leu Pro Ala Val
            340                 345                 350

Ala Asn Met Leu Leu Glu Val Gly Gly Leu Glu Phe Pro Gly Cys Pro
        355                 360                 365
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Phe Asn Gly Trp Tyr Met Gly Thr Glu Ile Gly Val Arg Asp Phe Cys
    370                 375                 380

Asp Val Gln Arg Tyr Asn Ile Leu Glu Glu Val Gly Arg Arg Met Gly
385                 390                 395                 400

Leu Glu Thr His Lys Leu Ala Ser Leu Trp Lys Asp Gln Ala Val Val
                405                 410                 415

Glu Ile Asn Ile Ala Val Ile His Ser Phe Gln Lys Gln Asn Val Thr
            420                 425                 430

Ile Met Asp His His Ser Ala Ala Glu Ser Phe Met Lys Tyr Met Gln
        435                 440                 445

Asn Glu Tyr Arg Ser Arg Gly Gly Cys Pro Ala Asp Trp Ile Trp Leu
    450                 455                 460

Val Pro Pro Met Ser Gly Ser Ile Thr Pro Val Phe His Gln Glu Met
465                 470                 475                 480

Leu Asn Tyr Val Leu Ser Pro Phe Tyr Tyr Tyr Gln Val Glu Ala Trp
                485                 490                 495

Lys Thr His Val Trp Gln Asp Glu Lys Arg Arg Pro Lys Arg Arg Glu
            500                 505                 510

Ile Pro Leu Lys Val Leu Val Lys Ala Val Leu Phe Ala Cys Met Leu
        515                 520                 525

Met Arg Lys Thr Met Ala Ser Arg Val Arg Val Thr Ile Leu Phe Ala
    530                 535                 540

Thr Glu Thr Gly Lys Ser Glu Ala Leu Ala Trp Asp Leu Gly Ala Leu
545                 550                 555                 560

Phe Ser Cys Ala Phe Asn Pro Lys Val Val Cys Met Asp Lys Tyr Arg
                565                 570                 575

Leu Ser Cys Leu Glu Glu Glu Arg Leu Leu Leu Val Val Thr Ser Thr
            580                 585                 590

Phe Gly Asn Gly Asp Cys Pro Gly Asn Gly Glu Lys Leu Lys Lys Ser
        595                 600                 605

Leu Phe Met Leu Lys Glu Leu Asn Asn Lys Phe Arg Tyr Ala Val Phe
    610                 615                 620

Gly Leu Gly Ser Ser Met Tyr Pro Arg Phe Cys Ala Phe Ala His Asp
625                 630                 635                 640

Ile Asp Gln Lys Leu Ser His Leu Gly Ala Ser Gln Leu Thr Pro Met
                645                 650                 655

Gly Glu Gly Asp Glu Leu Ser Gly Gln Glu Asp Ala Phe Arg Ser Trp
            660                 665                 670
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Ala Val Gln Thr Phe Lys Ala Ala Cys Glu Thr Phe Asp Val Arg Gly
        675                 680                 685

Lys Gln His Ile Gln Ile Pro Lys Leu Tyr Thr Ser Asn Val Thr Trp
    690                 695                 700

Asp Pro His His Tyr Arg Leu Val Gln Asp Ser Gln Pro Leu Asp Leu
705                 710                 715                 720

Ser Lys Ala Leu Ser Ser Met His Ala Lys Asn Val Phe Thr Met Arg
                725                 730                 735

Leu Lys Ser Arg Gln Asn Leu Gln Ser Pro Thr Ser Ser Arg Ala Thr
            740                 745                 750

Ile Leu Val Glu Leu Ser Cys Glu Asp Gly Gln Gly Leu Asn Tyr Leu
        755                 760                 765

Pro Gly Glu His Leu Gly Val Cys Pro Gly Asn Gln Pro Ala Leu Val
    770                 775                 780

Gln Gly Ile Leu Glu Arg Val Val Asp Gly Pro Thr Pro His Gln Thr
785                 790                 795                 800

Val Arg Leu Glu Asp Leu Asp Glu Ser Gly Ser Tyr Trp Val Ser Asp
                805                 810                 815

Lys Arg Leu Pro Pro Cys Ser Leu Ser Gln Ala Leu Thr Tyr Ser Pro
            820                 825                 830

Asp Ile Thr Thr Pro Pro Thr Gln Leu Leu Leu Gln Lys Leu Ala Gln
        835                 840                 845

Val Ala Thr Glu Glu Pro Glu Arg Gln Arg Leu Glu Ala Leu Cys Gln
    850                 855                 860

Pro Ser Glu Tyr Ser Lys Trp Lys Phe Thr Asn Ser Pro Thr Phe Leu
865                 870                 875                 880

Glu Val Leu Glu Glu Phe Pro Ser Leu Arg Val Ser Ala Gly Phe Leu
                885                 890                 895

Leu Ser Gln Leu Pro Ile Leu Lys Pro Arg Phe Tyr Ser Ile Ser Ser
            900                 905                 910

Ser Arg Asp His Thr Pro Thr Glu Ile His Leu Thr Val Ala Val Val
        915                 920                 925

Thr Tyr His Thr Gly Asp Gly Gln Gly Pro Leu His His Gly Val Cys
    930                 935                 940

Ser Thr Trp Leu Asn Ser Leu Lys Pro Gln Asp Pro Val Pro Cys Phe
945                 950                 955                 960

Val Arg Asn Ala Ser Ala Phe His Leu Pro Glu Asp Pro Ser His Pro
                965                 970                 975
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Cys Ile Leu Ile Gly Pro Gly Thr Gly Ile Val Pro Phe Arg Ser Phe
            980                 985                 990

Trp Gln Gln Arg Leu His Asp Ser Gln His Lys Gly Val Arg Gly Gly
        995                 1000                1005

Arg Met Thr Leu Val Phe Gly Cys Arg Arg Pro Asp Glu Asp His Ile
    1010                1015                1020

Tyr Gln Glu Glu Met Leu Glu Met Ala Gln Lys Gly Val Leu His Ala
1025                1030                1035                1040

Val His Thr Ala Tyr Ser Arg Leu Pro Gly Lys Pro Lys Val Tyr Val
                1045                1050                1055

Gln Asp Ile Leu Arg Gln Gln Leu Ala Ser Glu Val Leu Arg Val Leu
            1060                1065                1070

His Lys Glu Pro Gly His Leu Tyr Val Cys Gly Asp Val Arg Met Ala
        1075                1080                1085

Arg Asp Val Ala His Thr Leu Lys Gln Leu Val Ala Ala Lys Leu Lys
    1090                1095                1100

Leu Asn Glu Glu Gln Val Glu Asp Tyr Phe Phe Gln Leu Lys Ser Gln
1105                1110                1115                1120

Lys Arg Tyr His Glu Asp Ile Phe Gly Ala Val Phe Pro Tyr Glu Ala
                1125                1130                1135

Lys Lys Asp Arg Val Ala Val Gln Pro Ser Ser Leu Glu Met Ser Ala
            1140                1145                1150

Leu
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WHAT IS CLAIMED IS:

1. A process for preparing a cDNA clone coding for a human tissue inducible nitric oxide synthase comprising:

inducing said human tissue nitric oxide synthase in vitro;

identifying human tissue nitric oxide synthase mRNA by employing a cDNA probe capable of hybridizing with said human tissue inducible nitric oxide synthase mRNA;

isolating said human tissue nitric oxide synthase mRNA;

collecting said human tissue nitric oxide synthase mRNA;

separating human tissue poly A mRNA from said human tissue nitric oxide synthase mRNA;

constructing a human tissue inducible nitric oxide synthase cDNA library from said human tissue poly A mRNA using a reverse transcriptase enzyme and inserting a strand of said cDNA into a phage vector;

screening said cDNA library for human tissue inducible nitric oxide synthase clones including incubating said phage vector containing said cDNA with a bacteria for forming at least one positive plaque containing said cDNA clone for said human tissue inducible nitric oxide synthase;

rescuing said cDNA clone from said phage vector by employing a helper phage; and

converting said cDNA clone to a plasmid vector for obtaining a substantially full length cDNA clone encoding said human tissue inducible nitric oxide synthase.

2. A process of Claim 1 including excising cDNA inserts for human tissue inducible nitric oxide synthase from said plasmid vector.

3. A process of Claim 2 including confirming said cDNA inserts by employing a dideoxynucleotide DNA sequencing.

4. A process of Claim 2, including confirming said cDNA inserts by employing Southern blot hybridization.

5. A process of Claim 2 including expressing a human tissue inducible nitric oxide synthase protein by employing said cDNA

inserts for human tissue inducible nitric oxide synthase in an expression system.

6. A process of Claim 5 including employing said expression system being a baculovirus expression system.

7. A process of Claim 5 including purifying said human tissue inducible nitric oxide synthase protein.

8. A process of Claim 1 including employing said human tissue inducible nitric oxide synthase being a human hepatocyte inducible nitric oxide synthase.

9. A process of Claim 2 including employing said human tissue inducible nitric oxide synthase being a human hepatocyte inducible nitric oxide synthase.

10. A process of Claim 5 including employing said human tissue inducible nitric oxide synthase protein being a human hepatocyte inducible nitric oxide synthase protein.

11. A process of Claim 7 including employing said human tissue inducible nitric oxide synthase protein being a human hepatocyte inducible nitric oxide synthase protein.

12. A process of Claim 1 including inducing said human tissue nitric oxide synthase in vitro by stimulating a human tissue in vitro with at least one of the following (1) at least one cytokine, (2) at least one bacterial endotoxin and (3) combinations thereof.

13. A process of Claim 12 including inducing said human tissue nitric oxide synthase in vitro by stimulating a human tissue in vitro with at least one of the following (1) at least one cytokine comprising a cytokine selected from the group consisting of tissue necrosis factor, interleukin-1 and interferon-gamma, (2) at least one bacterial endotoxin including a bacterial lipopolysaccharide, and (3) combinations thereof.

14. A process of Claim 12 including identifying said human tissue nitric oxide synthase mRNA by employing a cDNA probe capable of hybridizing with said human tissue nitric oxide synthase mRNA.

15. A process of Claim 14 including inserting said cDNA strand having a length of about at least 1000 base pairs into said phage vector.

16. A process of Claim 15 including employing said phage vector being lambda Zap II.

17. A process of Claim 15 including screening said cDNA library including incubating said phage vector with a bacteria at a temperature from about 34 to 40 degrees centigrade for about 6 to 24 hours for effectuating phage lysis of said bacteria.

18. A process of Claim 17 including rescuing said cDNA clone from said phage vector by employing a helper phage.

19. A process of Claim 18 including employing said helper phage being ExAssist helper phage.

20. A process of Claim 18 including converting said rescued cDNA clone to said plasmid vector for obtaining said substantially full length cDNA clone encoding said human tissue inducible nitric oxide synthase, and employing pBluescript as said plasmid vector.

21. A process of Claim 20 including employing said human tissue inducible nitric oxide synthase being a human hepatocyte inducible nitric oxide synthase.

22. A human hepatocyte inducible nitric oxide synthase cDNA clone comprising a cDNA coding for the amino acid sequence, SEQ ID NO:2:

Met Ala Cys Pro Trp Lys Phe Leu Phe Lys Thr Lys Phe His Gln Tyr
Ala Met Asn Gly Glu Lys Asp Ile Asn Asn Asn Val Glu Lys Ala Pro
Cys Ala Thr Ser Ser Pro Val Thr Gln Asp Asp Leu Gln Tyr His Asn
Leu Ser Lys Gln Gln Asn Glu Ser Pro Gln Pro Leu Val Glu Thr Gly
Lys Lys Ser Pro Glu Ser Leu Val Lys Leu Asp Ala Thr Pro Leu Ser
Ser Pro Arg His Val Arg Ile Lys Asn Trp Gly Ser Gly Met Thr Phe
Gln Asp Thr Leu His His Lys Ala Lys Gly Ile Leu Thr Cys Arg Ser
Lys Ser Cys Leu Gly Ser Ile Met Thr Pro Lys Ser Leu Thr Arg Gly
Pro Arg Asp Lys Pro Thr Pro Pro Asp Glu Leu Leu Pro Gln Ala Ile
Glu Phe Val Asn Gln Tyr Tyr Gly Ser Phe Lys Glu Ala Lys Ile Glu
Glu His Leu Ala Arg Val Glu Ala Val Thr Lys Glu Ile Glu Thr Thr
Gly Thr Tyr Gln Leu Thr Gly Asp Glu Leu Ile Phe Ala Thr Lys Gln

Ala Trp Arg Asn Ala Pro Arg Cys Ile Gly Arg Ile Gln Trp Ser Asn
Leu Gln Val Phe Asp Ala Arg Ser Cys Ser Thr Ala Arg Glu Met Phe
Glu His Ile Cys Arg His Val Arg Tyr Ser Thr Asn Asn Gly Asn Ile
Arg Ser Ala Ile Thr Val Phe Pro Gln Arg Ser Asp Gly Lys His Asp
Phe Arg Val Trp Asn Ala Gln Leu Ile Arg Tyr Ala Gly Tyr Gln Met
Pro Asp Gly Ser Ile Arg Gly Asp Pro Ala Asn Val Glu Phe Thr Gln
Leu Cys Ile Asp Leu Gly Trp Lys Pro Lys Tyr Gly Arg Phe Asp Val
Val Pro Leu Val Leu Gln Ala Asn Gly Arg Asp Pro Glu Leu Phe Glu
Ile Pro Pro Asp Leu Val Leu Glu Val Ala Met Glu His Pro Lys Tyr
Glu Trp Phe Arg Glu Leu Glu Leu Lys Trp Tyr Ala Leu Pro Ala Val
Ala Asn Met Leu Leu Glu Val Gly Gly Leu Glu Phe Pro Gly Cys Pro
Phe Asn Gly Trp Tyr Met Gly Thr Glu Ile Gly Val Arg Asp Phe Cys
Asp Val Gln Arg Tyr Asn Ile Leu Glu Glu Val Gly Arg Arg Met Gly
Leu Glu Thr His Lys Leu Ala Ser Leu Trp Lys Asp Gln Ala Val Val
Glu Ile Asn Ile Ala Val Ile His Ser Phe Gln Lys Gln Asn Val Thr
Ile Met Asp His His Ser Ala Ala Glu Ser Phe Met Lys Tyr Met Gln
Asn Glu Tyr Arg Ser Arg Gly Gly Cys Pro Ala Asp Trp Ile Trp Leu
Val Pro Pro Met Ser Gly Ser Ile Thr Pro Val Phe His Gln Glu Met
Leu Asn Tyr Val Leu Ser Pro Phe Tyr Tyr Tyr Gln Val Glu Ala Trp
Lys Thr His Val Trp Gln Asp Glu Lys Arg Arg Pro Lys Arg Arg Glu
Ile Pro Leu Lys Val Leu Val Lys Ala Val Leu Phe Ala Cys Met Leu
Met Arg Lys Thr Met Ala Ser Arg Val Arg Val Thr Ile Leu Phe Ala
Thr Glu Thr Gly Lys Ser Glu Ala Leu Ala Trp Asp Leu Gly Ala Leu
Phe Ser Cys Ala Phe Asn Pro Lys Val Val Cys Met Asp Lys Tyr Arg
Leu Ser Cys Leu Glu Glu Glu Arg Leu Leu Leu Val Val Thr Ser Thr
Phe Gly Asn Gly Asp Cys Pro Gly Asn Gly Glu Lys Leu Lys Lys Ser
Leu Phe Met Leu Lys Glu Leu Asn Asn Lys Phe Arg Tyr Ala Val Phe

Gly Leu Gly Ser Ser Met Tyr Pro Arg Phe Cys Ala Phe Ala His Asp
Ile Asp Gln Lys Leu Ser His Leu Gly Ala Ser Gln Leu Thr Pro Met
Gly Glu Gly Asp Glu Leu Ser Gly Gln Glu Asp Ala Phe Arg Ser Trp
Ala Val Gln Thr Phe Lys Ala Ala Cys Glu Thr Phe Asp Val Arg Gly
Lys Gln His Ile Gln Ile Pro Lys Leu Tyr Thr Ser Asn Val Thr Trp
Asp Pro His His Tyr Arg Leu Val Gln Asp Ser Gln Pro Leu Asp Leu
Ser Lys Ala Leu Ser Ser Met His Ala Lys Asn Val Phe Thr Met Arg
Leu Lys Ser Arg Gln Asn Leu Gln Ser Pro Thr Ser Ser Arg Ala Thr
Ile Leu Val Glu Leu Ser Cys Glu Asp Gly Gln Gly Leu Asn Tyr Leu
Pro Gly Glu His Leu Gly Val Cys Pro Gly Asn Gln Pro Ala Leu Val
Gln Gly Ile Leu Glu Arg Val Val Asp Gly Pro Thr Pro His Gln Thr
Val Arg Leu Glu Asp Leu Asp Glu Ser Gly Ser Tyr Trp Val Ser Asp
Lys Arg Leu Pro Pro Cys Ser Leu Ser Gln Ala Leu Thr Tyr Ser Pro
Asp Ile Thr Thr Pro Pro Thr Gln Leu Leu Leu Gln Lys Leu Ala Gln
Val Ala Thr Glu Glu Pro Glu Arg Gln Arg Leu Glu Ala Leu Cys Gln
Pro Ser Glu Tyr Ser Lys Trp Lys Phe Thr Asn Ser Pro Thr Phe Leu
Glu Val Leu Glu Glu Phe Pro Ser Leu Arg Val Ser Ala Gly Phe Leu
Leu Ser Gln Leu Pro Ile Leu Lys Pro Arg Phe Tyr Ser Ile Ser Ser
Ser Arg Asp His Thr Pro Thr Glu Ile His Leu Thr Val Ala Val Val
Thr Tyr His Thr Gly Asp Gly Gln Gly Pro Leu His His Gly Val Cys
Ser Thr Trp Leu Asn Ser Leu Lys Pro Gln Asp Pro Val Pro Cys Phe
Val Arg Asn Ala Ser Ala Phe His Leu Pro Glu Asp Pro Ser His Pro
Cys Ile Leu Ile Gly Pro Gly Thr Gly Ile Val Pro Phe Arg Ser Phe
Trp Gln Gln Arg Leu His Asp Ser Gln His Lys Gly Val Arg Gly Gly
Arg Met Thr Leu Val Phe Gly Cys Arg Arg Pro Asp Glu Asp His Ile
Tyr Gln Glu Glu Met Leu Glu Met Ala Gln Lys Gly Val Leu His Ala
Val His Thr Ala Tyr Ser Arg Leu Pro Gly Lys Pro Lys Val Tyr Val

Gln Asp Ile Leu Arg Gln Gln Leu Ala Ser Glu Val Leu Arg Val Leu
His Lys Glu Pro Gly His Leu Tyr Val Cys Gly Asp Val Arg Met Ala
Arg Asp Val Ala His Thr Leu Lys Gln Leu Val Ala Ala Lys Leu Lys
Leu Asn Glu Glu Gln Val Glu Asp Tyr Phe Phe Gln Leu Lys Ser Gln
Lys Arg Tyr His Glu Asp Ile Phe Gly Ala Val Phe Pro Tyr Glu Ala
Lys Lys Asp Arg Val Ala Val Gln Pro Ser Ser Leu Glu Met Ser Ala
Leu

23. A human hepatocyte inducible nitric oxide synthase clone comprising the cDNA sequence, SEQ ID NO:1:

CTGCTTTAAA ATCTCTCGGC CACCTTTGAT GAGGGGACTG GGCAGTTCTA GACAGTCCCG
AAGTTCTCAA GGCACAGGTC TCTTCCTGGT TTGACTGTCC TTACCCCGGG GAGGCAGTGC
AGCCAGCTGC AAGCCCCACA GTGAAGAACA TCTGAGCTCA AATCCAGATA AGTGACATAA
GTGACCTGCT TTGTAAAGCC ATAGAG ATG GCC TGT CCT TGG AAA TTT CTG TTC
AAG ACC AAA TTC CAC CAG TAT GCA ATG AAT GGG GAA AAA GAC ATC AAC
AAC AAT GTG GAG AAA GCC CCC TGT GCC ACC TCC AGT CCA GTG ACA CAG
GAT GAC CTT CAG TAT CAC AAC CTC AGC AAG CAG CAG AAT GAG TCC CCG
CAG CCC CTC GTG GAG ACG GGA AAG AAG TCT CCA GAA TCT CTG GTC AAG
CTG GAT GCA ACC CCA TTG TCC TCC CCA CGG CAT GTG AGG ATC AAA AAC
TGG GGC AGC GGG ATG ACT TTC CAA GAC ACA CTT CAC CAT AAG GCC AAA
GGG ATT TTA ACT TGC AGG TCC AAA TCT TGC CTG GGG TCC ATT ATG ACT
CCC AAA AGT TTG ACC AGA GGA CCC AGG GAC AAG CCT ACC CCT CCA GAT
GAG CTT CTA CCT CAA GCT ATC GAA TTT GTC AAC CAA TAT TAC GGC TCC
TTC AAA GAG GCA AAA ATA GAG GAA CAT CTG GCC AGG GTG GAA GCG GTA
ACA AAG GAG ATA GAA ACA ACA GGA ACC TAC CAA CTG ACG GGA GAT GAG
CTC ATC TTC GCC ACC AAG CAG GCC TGG CGC AAT GCC CCA CGC TGC ATT
GGG AGG ATC CAG TGG TCC AAC CTG CAG GTC TTC GAT GCC CGC AGC TGT

TCC ACT GCC CGG GAA ATG TTT GAA CAC ATC TGC AGA CAC GTG CGT TAC

TCC ACC AAC AAT GGC AAC ATC AGG TCG GCC ATC ACC GTG TTC CCC CAG

CGG AGT GAT GGC AAG CAC GAC TTC CGG GTG TGG AAT GCT CAG CTC ATC

CGC TAT GCT GGC TAC CAG ATG CCA GAT GGC AGC ATC AGA GGG GAC CCT

GCC AAC GTG GAA TTC ACT CAG CTG TGC ATC GAC CTG GGC TGG AAG CCC

AAG TAC GGC CGC TTC GAT GTG GTC CCC CTG GTC CTG CAG GCC AAT GGC

CGT GAC CCT GAG CTC TTC GAA ATC CCA CCT GAC CTT GTG CTT GAG GTG

GCC ATG GAA CAT CCC AAA TAC GAG TGG TTT CGG GAA CTG GAG CTA AAG

TGG TAC GCC CTG CCT GCA GTG GCC AAC ATG CTG CTT GAG GTG GGC GGC

CTG GAG TTC CCA GGG TGC CCC TTC AAT GGC TGG TAC ATG GGC ACA GAG

ATC GGA GTC CGG GAC TTC TGT GAC GTC CAG CGC TAC AAC ATC CTG GAG

GAA GTG GGC AGG AGA ATG GGC CTG GAA ACG CAC AAG CTG GCC TCG CTC

TGG AAA GAC CAG GCT GTC GTT GAG ATC AAC ATT GCT GTG ATC CAT AGT

TTT CAG AAG CAG AAT GTG ACC ATC ATG GAC CAC CAC TCG GCT GCA GAA

TCC TTC ATG AAG TAC ATG CAG AAT GAA TAC CGG TCC CGT GGG GGC TGC

CCG GCA GAC TGG ATT TGG CTG GTC CCT CCC ATG TCT GGG AGC ATC ACC

CCC GTG TTT CAC CAG GAG ATG CTG AAC TAC GTC CTG TCC CCT TTC TAC

TAC TAT CAG GTA GAG GCC TGG AAA ACC CAT GTC TGG CAG GAC GAG AAG

CGG AGA CCC AAG AGA AGA GAG ATT CCA TTG AAA GTC TTG GTC AAA GCT

GTG CTC TTT GCC TGT ATG CTG ATG CGC AAG ACA ATG GCG TCC CGA GTC

AGA GTC ACC ATC CTC TTT GCG ACA GAG ACA GGA AAA TCA GAG GCG CTG

GCC TGG GAC CTG GGG GCC TTA TTC AGC TGT GCC TTC AAC CCC AAG GTT

GTC TGC ATG GAT AAG TAC AGG CTG AGC TGC CTG GAG GAG GAA CGG CTG

CTG TTG GTG GTG ACC AGT ACG TTT GGC AAT GGA GAC TGC CCT GGC AAT

GGA GAG AAA CTG AAG AAA TCG CTC TTC ATG CTG AAA GAG CTC AAC AAC

AAA TTC AGG TAC GCT GTG TTT GGC CTC GGC TCC AGC ATG TAC CCT CGG

TTC TGC GCC TTT GCT CAT GAC ATT GAT CAG AAG CTG TCC CAC CTG GGG

GCC TCT CAG CTC ACC CCG ATG GGA GAA GGG GAT GAG CTC AGT GGG CAG

GAG GAC GCC TTC CGC AGC TGG GCC GTG CAA ACC TTC AAG GCA GCC TGT

GAG ACG TTT GAT GTC CGA GGC AAA CAG CAC ATT CAG ATC CCC AAG CTC

TAC ACC TCC AAT GTG ACC TGG GAC CCG CAC CAC TAC AGG CTC GTG CAG

GAC TCA CAG CCT TTG GAC CTC AGC AAA GCC CTC AGC AGC ATG CAT GCC

AAG AAC GTG TTC ACC ATG AGG CTC AAA TCT CGG CAG AAT CTA CAA AGT

CCG ACA TCC AGC CGT GCC ACC ATC CTG GTG GAA CTC TCC TGT GAG GAT

GGC CAA GGC CTG AAC TAC CTG CCG GGG GAG CAC CTT GGG GTT TGC CCA

GGC AAC CAG CCG GCC CTG GTC CAA GGC ATC CTG GAG CGA GTG GTG GAT

GGC CCC ACA CCC CAC CAG ACA GTG CGC CTG GAG GAC CTG GAT GAG AGT

GGC AGC TAC TGG GTC AGT GAC AAG AGG CTG CCC CCC TGC TCA CTC AGC

CAG GCC CTC ACC TAC TCC CCG GAC ATC ACC ACA CCC CCA ACC CAG CTG

CTG CTC CAA AAG CTG GCC CAG GTG GCC ACA GAA GAG CCT GAG AGA CAG

AGG CTG GAG GCC CTG TGC CAG CCC TCA GAG TAC AGC AAG TGG AAG TTC

ACC AAC AGC CCC ACA TTC CTG GAG GTG CTA GAG GAG TTC CCG TCC CTG

CGG GTG TCT GCT GGC TTC CTG CTT TCC CAG CTC CCC ATT CTG AAG CCC

AGG TTC TAC TCC ATC AGC TCC TCC CGG GAT CAC ACG CCC ACG GAG ATC

CAC CTG ACT GTG GCC GTG GTC ACC TAC CAC ACC GGA GAT GGC CAG GGT

CCC CTG CAC CAC GGT GTC TGC AGC ACA TGG CTC AAC AGC CTG AAG CCC

CAA GAC CCA GTG CCC TGC TTT GTG CGG AAT GCC AGC GCC TTC CAC CTC

CCC GAG GAT CCC TCC CAT CCT TGC ATC CTC ATC GGG CCT GGC ACA GGC

ATC GTG CCC TTC CGC AGT TTC TGG CAG CAA CGG CTC CAT GAC TCC CAG

CAC AAG GGA GTG CGG GGA GGC CGC ATG ACC TTG GTG TTT GGG TGC CGC

CGC CCA GAT GAG GAC CAC ATC TAC CAG GAG GAG ATG CTG GAG ATG GCC

CAG AAG GGG GTG CTG CAT GCG GTG CAC ACA GCC TAT TCC CGC CTG CCT

GGC AAG CCC AAG GTC TAT GTT CAG GAC ATC CTG CGG CAG CAG CTG GCC
AGC GAG GTG CTC CGT GTG CTC CAC AAG GAG CCA GGC CAC CTC TAT GTT
TGC GGG GAT GTG CGC ATG GCC CGG GAC GTG GCC CAC ACC CTG AAG CAG
CTG GTG GCT GCC AAG CTG AAA TTG AAT GAG GAG CAG GTC GAG GAC TAT
TTC TTT CAG CTC AAG AGC CAG AAG CGC TAT CAC GAA GAT ATC TTC GGT
GCT GTA TTT CCT TAC GAG GCG AAG AAG GAC AGG GTG GCG GTG CAG CCC
AGC AGC CTG GAG ATG TCA GCG CTC TGAGGGCCTA CAGGAGGGGT TAAAGCTGCC
GGCACAGAAC TTAAGGATGG AGCCAGCTCT GCATTATCTG AGGTCACAGG GCCTGGGGAG
ATGGAGGAAA GTGATATCCC CCAGCCTCAA GTCTTATTTC CTCAACGTTG CTCCCCATCA
AGCCCTTTAC TTGACCTCCT AACAAGTAGC ACCCTGGATT GATCGGAGCC TCCTCTCTCA
AACTGGGGCC TCCCTGGTCC CTTGGAGACA AAATCTTAAA TGCCAGGCCT GGCGAGTGGG
TGAAAGATGG AACTTGCTGC TGAGTGCACC ACTTCAAGTG ACCACCAGGA GGTGCTATCG
CACCACTGTG TATTTAACTG CCTTGTGTAC AGTTATTTAT GCCTCTGTAT TTAAAAAACT
AACACCCAGT CTGTTCCCCA TGGCCACTTG GGTCTTCCCT GTATGATTCC TTGATGGAGA
TATTTACATG AATTGCATTT TACTTTAATC

24. A process for producing human hepatocyte inducible nitric oxide synthase protein comprising:

providing a replicatable DNA expression vector capable of expressing a DNA sequence encoding human hepatocyte inducible nitric oxide synthase in a suitable host;

transforming said host for obtaining a recombinant host; and

maintaining said recombinant host under conditions permitting expression of said DNA sequence to provide said human hepatocyte inducible nitric oxide synthase.

25. A recombinant protein comprising a human tissue inducible nitric oxide synthase recombinant protein expressed from a human tissue inducible nitric oxide synthase cDNA clone.

26. A recombinant protein comprising a human hepatocyte inducible nitric oxide synthase recombinant protein expressed from a human hepatocyte inducible nitric oxide synthase cDNA clone.

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CTGCTTTAAA ATCTCTCGGC CACCTTTGAT GAGGGGACTG GGCAGTTCTA GACAGTCCCG      60

AAGTTCTCAA GGCACAGGTC TCTTCCTGGT TTGACTGTCC TTACCCCGGG GAGGCAGTGC     120

AGCCAGCTGC AAGCCCCACA GTGAAGAACA TCTGAGCTCA AATCCAGATA AGTGACATAA     180

GTGACCTGCT TTGTAAAGCC ATAGAG ATG GCC TGT CCT TGG AAA TTT CTG TTC       233
                             Met Ala Cys Pro Trp Lys Phe Leu Phe
                               1               5

AAG ACC AAA TTC CAC CAG TAT GCA ATG AAT GGG GAA AAA GAC ATC AAC       281
Lys Thr Lys Phe His Gln Tyr Ala Met Asn Gly Glu Lys Asp Ile Asn
 10                  15                  20                  25

AAC AAT GTG GAG AAA GCC CCC TGT GCC ACC TCC AGT CCA GTG ACA CAG       329
Asn Asn Val Glu Lys Ala Pro Cys Ala Thr Ser Ser Pro Val Thr Gln
                 30                  35                  40

GAT GAC CTT CAG TAT CAC AAC CTC AGC AAG CAG CAG AAT GAG TCC CCG       377
Asp Asp Leu Gln Tyr His Asn Leu Ser Lys Gln Gln Asn Glu Ser Pro
             45                  50                  55

CAG CCC CTC GTG GAG ACG GGA AAG AAG TCT CCA GAA TCT CTG GTC AAG       425
Gln Pro Leu Val Glu Thr Gly Lys Lys Ser Pro Glu Ser Leu Val Lys
         60                  65                  70

CTG GAT GCA ACC CCA TTG TCC TCC CCA CGG CAT GTG AGG ATC AAA AAC       473
Leu Asp Ala Thr Pro Leu Ser Ser Pro Arg His Val Arg Ile Lys Asn
     75                  80                  85

TGG GGC AGC GGG ATG ACT TTC CAA GAC ACA CTT CAC CAT AAG GCC AAA       521
Trp Gly Ser Gly Met Thr Phe Gln Asp Thr Leu His His Lys Ala Lys
 90                  95                 100                 105

GGG ATT TTA ACT TGC AGG TCC AAA TCT TGC CTG GGG TCC ATT ATG ACT       569
Gly Ile Leu Thr Cys Arg Ser Lys Ser Cys Leu Gly Ser Ile Met Thr
                110                 115                 120

CCC AAA AGT TTG ACC AGA GGA CCC AGG GAC AAG CCT ACC CCT CCA GAT       617
Pro Lys Ser Leu Thr Arg Gly Pro Arg Asp Lys Pro Thr Pro Pro Asp
            125                 130                 135
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FIGURE 1A

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GAG CTT CTA CCT CAA GCT ATC GAA TTT GTC AAC CAA TAT TAC GGC TCC      665
Glu Leu Leu Pro Gln Ala Ile Glu Phe Val Asn Gln Tyr Tyr Gly Ser
        140                 145                 150

TTC AAA GAG GCA AAA ATA GAG GAA CAT CTG GCC AGG GTG GAA GCG GTA      713
Phe Lys Glu Ala Lys Ile Glu Glu His Leu Ala Arg Val Glu Ala Val
    155                 160                 165

ACA AAG GAG ATA GAA ACA ACA GGA ACC TAC CAA CTG ACG GGA GAT GAG      761
Thr Lys Glu Ile Glu Thr Thr Gly Thr Tyr Gln Leu Thr Gly Asp Glu
170                 175                 180                 185

CTC ATC TTC GCC ACC AAG CAG GCC TGG CGC AAT GCC CCA CGC TGC ATT      809
Leu Ile Phe Ala Thr Lys Gln Ala Trp Arg Asn Ala Pro Arg Cys Ile
                190                 195                 200

GGG AGG ATC CAG TGG TCC AAC CTG CAG GTC TTC GAT GCC CGC AGC TGT      857
Gly Arg Ile Gln Trp Ser Asn Leu Gln Val Phe Asp Ala Arg Ser Cys
            205                 210                 215

TCC ACT GCC CGG GAA ATG TTT GAA CAC ATC TGC AGA CAC GTG CGT TAC      905
Ser Thr Ala Arg Glu Met Phe Glu His Ile Cys Arg His Val Arg Tyr
        220                 225                 230

TCC ACC AAC AAT GGC AAC ATC AGG TCG GCC ATC ACC GTG TTC CCC CAG      953
Ser Thr Asn Asn Gly Asn Ile Arg Ser Ala Ile Thr Val Phe Pro Gln
    235                 240                 245

CGG AGT GAT GGC AAG CAC GAC TTC CGG GTG TGG AAT GCT CAG CTC ATC     1001
Arg Ser Asp Gly Lys His Asp Phe Arg Val Trp Asn Ala Gln Leu Ile
250                 255                 260                 265

CGC TAT GCT GGC TAC CAG ATG CCA GAT GGC AGC ATC AGA GGG GAC CCT     1049
Arg Tyr Ala Gly Tyr Gln Met Pro Asp Gly Ser Ile Arg Gly Asp Pro
                270                 275                 280

GCC AAC GTG GAA TTC ACT CAG CTG TGC ATC GAC CTG GGC TGG AAG CCC     1097
Ala Asn Val Glu Phe Thr Gln Leu Cys Ile Asp Leu Gly Trp Lys Pro
            285                 290                 295

AAG TAC GGC CGC TTC GAT GTG GTC CCC CTG GTC CTG CAG GCC AAT GGC     1145
Lys Tyr Gly Arg Phe Asp Val Val Pro Leu Val Leu Gln Ala Asn Gly
        300                 305                 310
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FIGURE 1B

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CGT GAC CCT GAG CTC TTC GAA ATC CCA CCT GAC CTT GTG CTT GAG GTG      1193
Arg Asp Pro Glu Leu Phe Glu Ile Pro Pro Asp Leu Val Leu Glu Val
    315                 320                 325

GCC ATG GAA CAT CCC AAA TAC GAG TGG TTT CGG GAA CTG GAG CTA AAG      1241
Ala Met Glu His Pro Lys Tyr Glu Trp Phe Arg Glu Leu Glu Leu Lys
330                 335                 340                 345

TGG TAC GCC CTG CCT GCA GTG GCC AAC ATG CTG CTT GAG GTG GGC GGC      1289
Trp Tyr Ala Leu Pro Ala Val Ala Asn Met Leu Leu Glu Val Gly Gly
                350                 355                 360

CTG GAG TTC CCA GGG TGC CCC TTC AAT GGC TGG TAC ATG GGC ACA GAG      1337
Leu Glu Phe Pro Gly Cys Pro Phe Asn Gly Trp Tyr Met Gly Thr Glu
            365                 370                 375

ATC GGA GTC CGG GAC TTC TGT GAC GTC CAG CGC TAC AAC ATC CTG GAG      1385
Ile Gly Val Arg Asp Phe Cys Asp Val Gln Arg Tyr Asn Ile Leu Glu
        380                 385                 390

GAA GTG GGC AGG AGA ATG GGC CTG GAA ACG CAC AAG CTG GCC TCG CTC      1433
Glu Val Gly Arg Arg Met Gly Leu Glu Thr His Lys Leu Ala Ser Leu
    395                 400                 405

TGG AAA GAC CAG GCT GTC GTT GAG ATC AAC ATT GCT GTG ATC CAT AGT      1481
Trp Lys Asp Gln Ala Val Val Glu Ile Asn Ile Ala Val Ile His Ser
410                 415                 420                 425

TTT CAG AAG CAG AAT GTG ACC ATC ATG GAC CAC CAC TCG GCT GCA GAA      1529
Phe Gln Lys Gln Asn Val Thr Ile Met Asp His His Ser Ala Ala Glu
                430                 435                 440

TCC TTC ATG AAG TAC ATG CAG AAT GAA TAC CGG TCC CGT GGG GGC TGC      1577
Ser Phe Met Lys Tyr Met Gln Asn Glu Tyr Arg Ser Arg Gly Gly Cys
            445                 450                 455

CCG GCA GAC TGG ATT TGG CTG GTC CCT CCC ATG TCT GGG AGC ATC ACC      1625
Pro Ala Asp Trp Ile Trp Leu Val Pro Pro Met Ser Gly Ser Ile Thr
        460                 465                 470

CCC GTG TTT CAC CAG GAG ATG CTG AAC TAC GTC CTG TCC CCT TTC TAC      1673
Pro Val Phe His Gln Glu Met Leu Asn Tyr Val Leu Ser Pro Phe Tyr
    475                 480                 485
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FIGURE 1C

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TAC TAT CAG GTA GAG GCC TGG AAA ACC CAT GTC TGG CAG GAC GAG AAG      1721
Tyr Tyr Gln Val Glu Ala Trp Lys Thr His Val Trp Gln Asp Glu Lys
490                 495                 500                 505

CGG AGA CCC AAG AGA AGA GAG ATT CCA TTG AAA GTC TTG GTC AAA GCT      1769
Arg Arg Pro Lys Arg Arg Glu Ile Pro Leu Lys Val Leu Val Lys Ala
                510                 515                 520

GTG CTC TTT GCC TGT ATG CTG ATG CGC AAG ACA ATG GCG TCC CGA GTC      1817
Val Leu Phe Ala Cys Met Leu Met Arg Lys Thr Met Ala Ser Arg Val
            525                 530                 535

AGA GTC ACC ATC CTC TTT GCG ACA GAG ACA GGA AAA TCA GAG GCG CTG      1865
Arg Val Thr Ile Leu Phe Ala Thr Glu Thr Gly Lys Ser Glu Ala Leu
        540                 545                 550

GCC TGG GAC CTG GGG GCC TTA TTC AGC TGT GCC TTC AAC CCC AAG GTT      1913
Ala Trp Asp Leu Gly Ala Leu Phe Ser Cys Ala Phe Asn Pro Lys Val
    555                 560                 565

GTC TGC ATG GAT AAG TAC AGG CTG AGC TGC CTG GAG GAG GAA CGG CTG      1961
Val Cys Met Asp Lys Tyr Arg Leu Ser Cys Leu Glu Glu Glu Arg Leu
570                 575                 580                 585

CTG TTG GTG GTG ACC AGT ACG TTT GGC AAT GGA GAC TGC CCT GGC AAT      2009
Leu Leu Val Val Thr Ser Thr Phe Gly Asn Gly Asp Cys Pro Gly Asn
                590                 595                 600

GGA GAG AAA CTG AAG AAA TCG CTC TTC ATG CTG AAA GAG CTC AAC AAC      2057
Gly Glu Lys Leu Lys Lys Ser Leu Phe Met Leu Lys Glu Leu Asn Asn
            605                 610                 615

AAA TTC AGG TAC GCT GTG TTT GGC CTC GGC TCC AGC ATG TAC CCT CGG      2105
Lys Phe Arg Tyr Ala Val Phe Gly Leu Gly Ser Ser Met Tyr Pro Arg
        620                 625                 630

TTC TGC GCC TTT GCT CAT GAC ATT GAT CAG AAG CTG TCC CAC CTG GGG      2153
Phe Cys Ala Phe Ala His Asp Ile Asp Gln Lys Leu Ser His Leu Gly
    635                 640                 645

GCC TCT CAG CTC ACC CCG ATG GGA GAA GGG GAT GAG CTC AGT GGG CAG      2201
Ala Ser Gln Leu Thr Pro Met Gly Glu Gly Asp Glu Leu Ser Gly Gln
650                 655                 660                 665

GAG GAC GCC TTC CGC AGC TGG GCC GTG CAA ACC TTC AAG GCA GCC TGT      2249
Glu Asp Ala Phe Arg Ser Trp Ala Val Gln Thr Phe Lys Ala Ala Cys
                670                 675                 680
```

FIGURE 1D

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GAG ACG TTT GAT GTC CGA GGC AAA CAG CAC ATT CAG ATC CCC AAG CTC      2297
Glu Thr Phe Asp Val Arg Gly Lys Gln His Ile Gln Ile Pro Lys Leu
            685                 690                 695

TAC ACC TCC AAT GTG ACC TGG GAC CCG CAC CAC TAC AGG CTC GTG CAG      2345
Tyr Thr Ser Asn Val Thr Trp Asp Pro His His Tyr Arg Leu Val Gln
        700                 705                 710

GAC TCA CAG CCT TTG GAC CTC AGC AAA GCC CTC AGC AGC ATG CAT GCC      2393
Asp Ser Gln Pro Leu Asp Leu Ser Lys Ala Leu Ser Ser Met His Ala
    715                 720                 725

AAG AAC GTG TTC ACC ATG AGG CTC AAA TCT CGG CAG AAT CTA CAA AGT      2441
Lys Asn Val Phe Thr Met Arg Leu Lys Ser Arg Gln Asn Leu Gln Ser
730                 735                 740                 745

CCG ACA TCC AGC CGT GCC ACC ATC CTG GTG GAA CTC TCC TGT GAG GAT      2489
Pro Thr Ser Ser Arg Ala Thr Ile Leu Val Glu Leu Ser Cys Glu Asp
                750                 755                 760

GGC CAA GGC CTG AAC TAC CTG CCG GGG GAG CAC CTT GGG GTT TGC CCA      2537
Gly Gln Gly Leu Asn Tyr Leu Pro Gly Glu His Leu Gly Val Cys Pro
            765                 770                 775

GGC AAC CAG CCG GCC CTG GTC CAA GGC ATC CTG GAG CGA GTG GTG GAT      2585
Gly Asn Gln Pro Ala Leu Val Gln Gly Ile Leu Glu Arg Val Val Asp
        780                 785                 790

GGC CCC ACA CCC CAC CAG ACA GTG CGC CTG GAG GAC CTG GAT GAG AGT      2633
Gly Pro Thr Pro His Gln Thr Val Arg Leu Glu Asp Leu Asp Glu Ser
    795                 800                 805

GGC AGC TAC TGG GTC AGT GAC AAG AGG CTG CCC CCC TGC TCA CTC AGC      2681
Gly Ser Tyr Trp Val Ser Asp Lys Arg Leu Pro Pro Cys Ser Leu Ser
810                 815                 820                 825

CAG GCC CTC ACC TAC TCC CCG GAC ATC ACC ACA CCC CCA ACC CAG CTG      2729
Gln Ala Leu Thr Tyr Ser Pro Asp Ile Thr Thr Pro Pro Thr Gln Leu
                830                 835                 840

CTG CTC CAA AAG CTG GCC CAG GTG GCC ACA GAA GAG CCT GAG AGA CAG      2777
Leu Leu Gln Lys Leu Ala Gln Val Ala Thr Glu Glu Pro Glu Arg Gln
            845                 850                 855
```

FIGURE 1E

```
AGG CTG GAG GCC CTG TGC CAG CCC TCA GAG TAC AGC AAG TGG AAG TTC      2825
Arg Leu Glu Ala Leu Cys Gln Pro Ser Glu Tyr Ser Lys Trp Lys Phe
        860                 865                 870

ACC AAC AGC CCC ACA TTC CTG GAG GTG CTA GAG GAG TTC CCG TCC CTG      2873
Thr Asn Ser Pro Thr Phe Leu Glu Val Leu Glu Glu Phe Pro Ser Leu
    875                 880                 885

CGG GTG TCT GCT GGC TTC CTG CTT TCC CAG CTC CCC ATT CTG AAG CCC      2921
Arg Val Ser Ala Gly Phe Leu Leu Ser Gln Leu Pro Ile Leu Lys Pro
890                 895                 900                 905

AGG TTC TAC TCC ATC AGC TCC TCC CGG GAT CAC ACG CCC ACG GAG ATC      2969
Arg Phe Tyr Ser Ile Ser Ser Ser Arg Asp His Thr Pro Thr Glu Ile
                910                 915                 920

CAC CTG ACT GTG GCC GTG GTC ACC TAC CAC ACC GGA GAT GGC CAG GGT      3017
His Leu Thr Val Ala Val Val Thr Tyr His Thr Gly Asp Gly Gln Gly
            925                 930                 935

CCC CTG CAC CAC GGT GTC TGC AGC ACA TGG CTC AAC AGC CTG AAG CCC      3065
Pro Leu His His Gly Val Cys Ser Thr Trp Leu Asn Ser Leu Lys Pro
        940                 945                 950

CAA GAC CCA GTG CCC TGC TTT GTG CGG AAT GCC AGC GCC TTC CAC CTC      3113
Gln Asp Pro Val Pro Cys Phe Val Arg Asn Ala Ser Ala Phe His Leu
    955                 960                 965

CCC GAG GAT CCC TCC CAT CCT TGC ATC CTC ATC GGG CCT GGC ACA GGC      3161
Pro Glu Asp Pro Ser His Pro Cys Ile Leu Ile Gly Pro Gly Thr Gly
970                 975                 980                 985

ATC GTG CCC TTC CGC AGT TTC TGG CAG CAA CGG CTC CAT GAC TCC CAG      3209
Ile Val Pro Phe Arg Ser Phe Trp Gln Gln Arg Leu His Asp Ser Gln
                990                 995                1000

CAC AAG GGA GTG CGG GGA GGC CGC ATG ACC TTG GTG TTT GGG TGC CGC      3257
His Lys Gly Val Arg Gly Gly Arg Met Thr Leu Val Phe Gly Cys Arg
            1005                1010                1015

CGC CCA GAT GAG GAC CAC ATC TAC CAG GAG GAG ATG CTG GAG ATG GCC      3305
Arg Pro Asp Glu Asp His Ile Tyr Gln Glu Glu Met Leu Glu Met Ala
        1020                1025                1030

CAG AAG GGG GTG CTG CAT GCG GTG CAC ACA GCC TAT TCC CGC CTG CCT      3353
Gln Lys Gly Val Leu His Ala Val His Thr Ala Tyr Ser Arg Leu Pro
    1035                1040                1045
```

FIGURE 1F

```
GGC AAG CCC AAG GTC TAT GTT CAG GAC ATC CTG CGG CAG CAG CTG GCC      3401
Gly Lys Pro Lys Val Tyr Val Gln Asp Ile Leu Arg Gln Gln Leu Ala
1050                1055                1060                1065

AGC GAG GTG CTC CGT GTG CTC CAC AAG GAG CCA GGC CAC CTC TAT GTT      3449
Ser Glu Val Leu Arg Val Leu His Lys Glu Pro Gly His Leu Tyr Val
                1070                1075                1080

TGC GGG GAT GTG CGC ATG GCC CGG GAC GTG GCC CAC ACC CTG AAG CAG      3497
Cys Gly Asp Val Arg Met Ala Arg Asp Val Ala His Thr Leu Lys Gln
            1085                1090                1095

CTG GTG GCT GCC AAG CTG AAA TTG AAT GAG GAG CAG GTC GAG GAC TAT      3545
Leu Val Ala Ala Lys Leu Lys Leu Asn Glu Glu Gln Val Glu Asp Tyr
        1100                1105                1110

TTC TTT CAG CTC AAG AGC CAG AAG CGC TAT CAC GAA GAT ATC TTC GGT      3593
Phe Phe Gln Leu Lys Ser Gln Lys Arg Tyr His Glu Asp Ile Phe Gly
    1115                1120                1125

GCT GTA TTT CCT TAC GAG GCG AAG AAG GAC AGG GTG GCG GTG CAG CCC      3641
Ala Val Phe Pro Tyr Glu Ala Lys Lys Asp Arg Val Ala Val Gln Pro
1130                1135                1140                1145

AGC AGC CTG GAG ATG TCA GCG CTC TGAGGGCCTA CAGGAGGGGT TAAAGCTGCC     3695
Ser Ser Leu Glu Met Ser Ala Leu
                1150

GGCACAGAAC TTAAGGATGG AGCCAGCTCT GCATTATCTG AGGTCACAGG GCCTGGGGAG    3755

ATGGAGGAAA GTGATATCCC CCAGCCTCAA GTCTTATTTC CTCAACGTTG CTCCCCATCA    3815

AGCCCTTTAC TTGACCTCCT AACAAGTAGC ACCCTGGATT GATCGGAGCC TCCTCTCTCA    3875

AACTGGGGCC TCCCTGGTCC CTTGGAGACA AAATCTTAAA TGCCAGGCCT GGCGAGTGGG    3935

TGAAAGATGG AACTTGCTGC TGAGTGCACC ACTTCAAGTG ACCACCAGGA GGTGCTATCG    3995

CACCACTGTG TATTTAACTG CCTTGTGTAC AGTTATTTAT GCCTCTGTAT TTAAAAAACT    4055

AACACCCAGT CTGTTCCCCA TGGCCACTTG GGTCTTCCCT GTATGATTCC TTGATGGAGA    4115

TATTTACATG AATTGCATTT TACTTTAATC                                     4145
```

FIGURE 1G

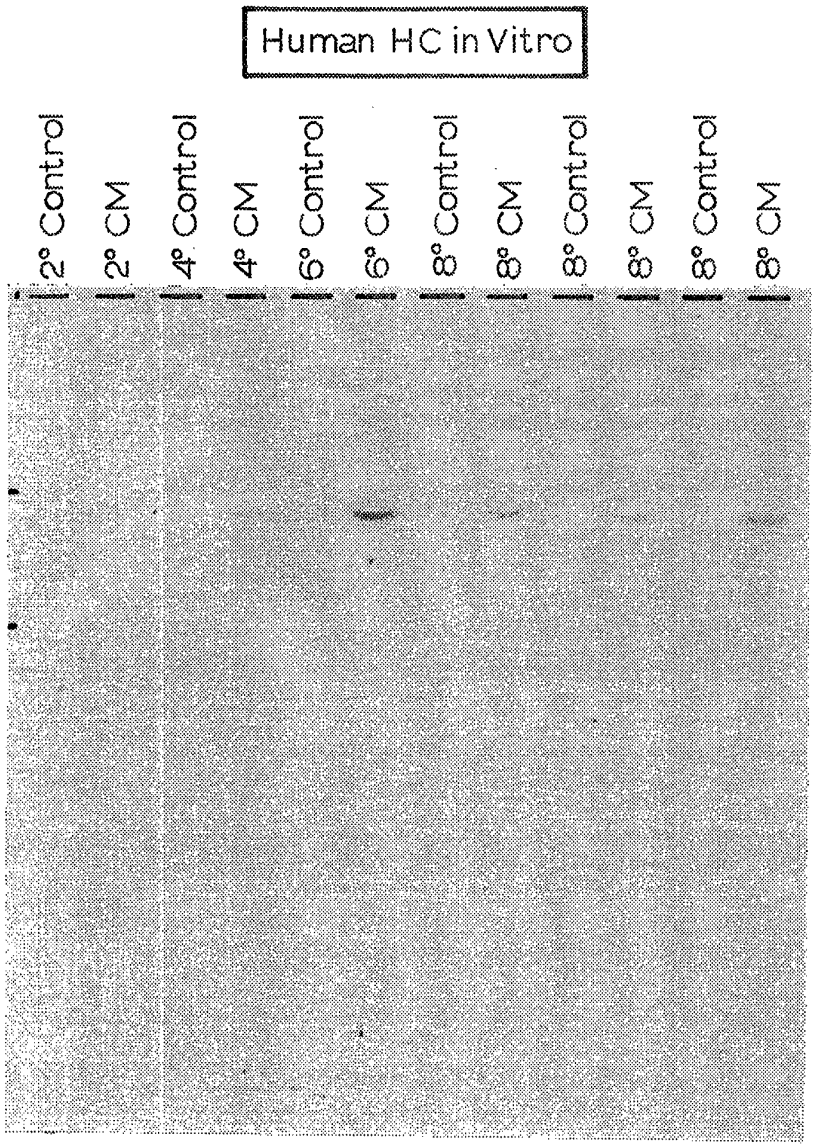
Human HC in Vitro
2° Control
2° CM
4° Control
4° CM
6° Control
6° CM
8° Control
8° CM
8° Control
8° CM
8° Control
8° CM
NB 25 Mac NOS insert
18° exposure
2/28/92

Fig. 2.

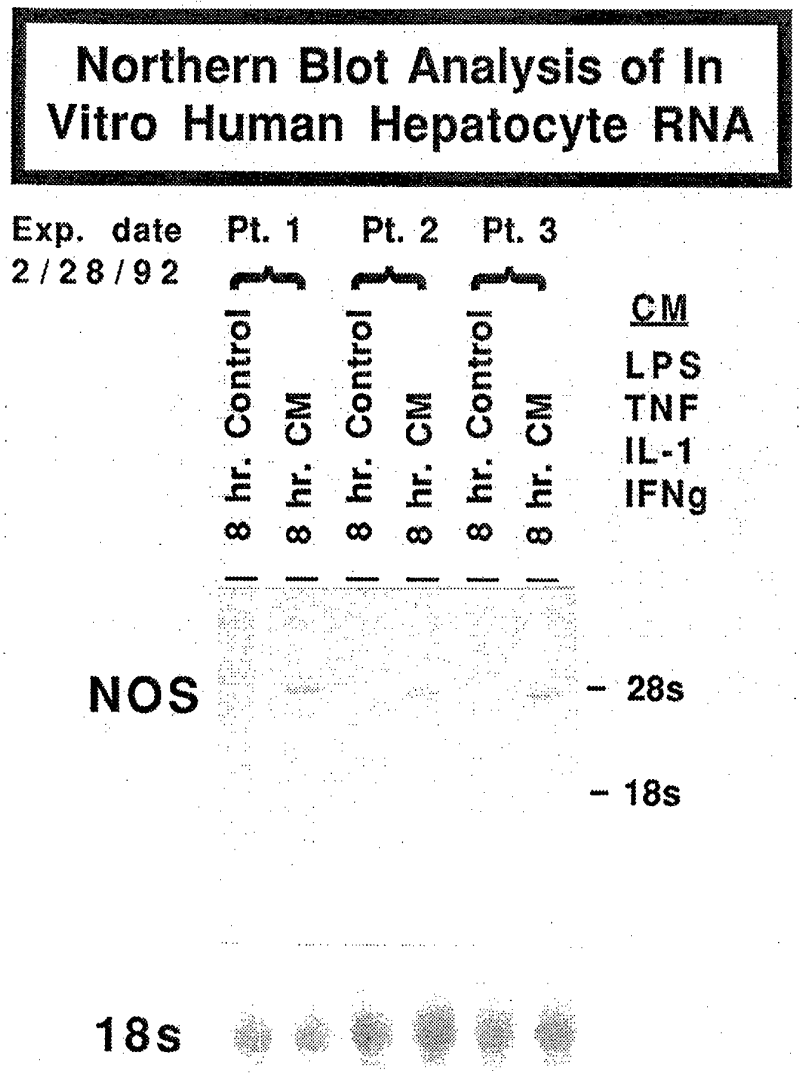
Northern Blot Analysis of In Vitro Human Hepatocyte RNA
Exp. date
2/28/92
Pt. 1
Pt. 2
Pt. 3
8 hr. Control
8 hr. CM
8 hr. Control
8 hr. CM
8 hr. Control
8 hr. CM
CM
LPS
TNF
IL-1
IFNg
NOS
28s
18s
18s

Fig. 3.

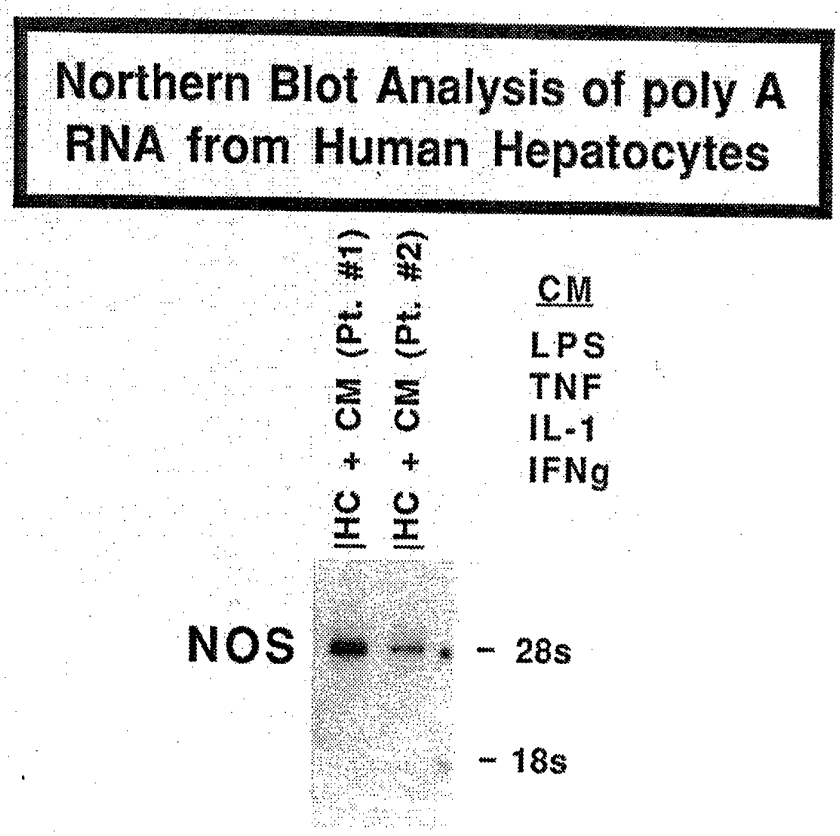
Northern Blot Analysis of poly A RNA from Human Hepatocytes
IHC + CM (Pt. #1)
IHC + CM (Pt. #2)
CM
LPS
TNF
IL-1
IFNg
NOS
- 28s
- 18s

Fig. 4.

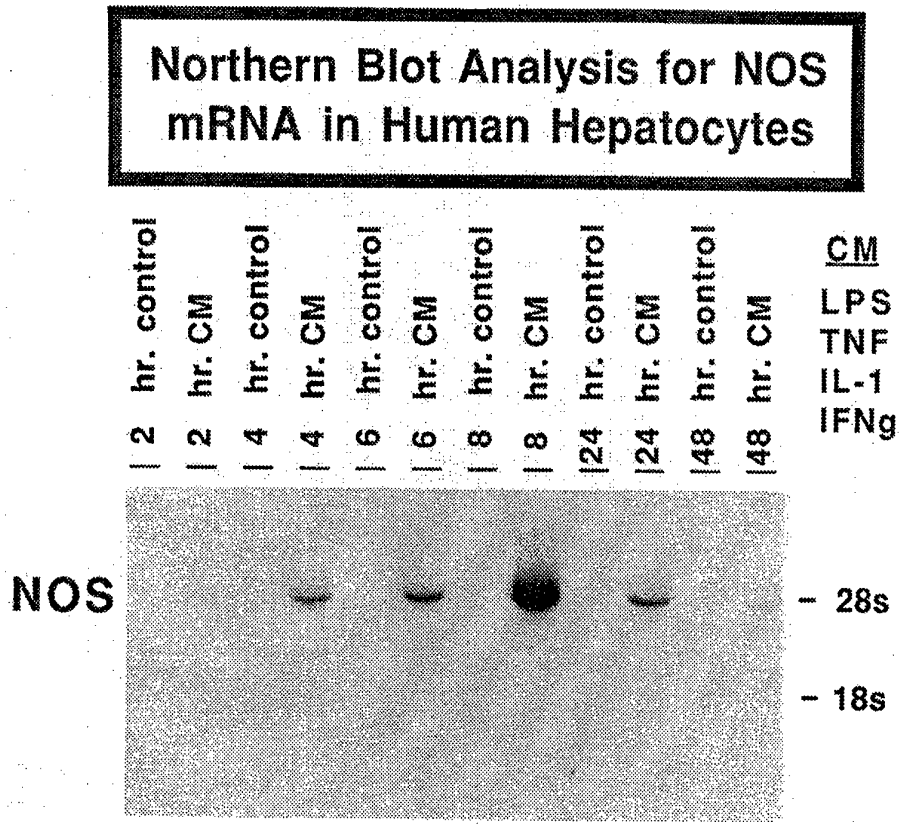
Northern Blot Analysis for NOS mRNA in Human Hepatocytes
2 hr. control
2 hr. CM
4 hr. control
4 hr. CM
6 hr. control
6 hr. CM
8 hr. control
8 hr. CM
24 hr. control
24 hr. CM
48 hr. control
48 hr. CM
CM
LPS
TNF
IL-1
IFNg
NOS
28s
18s

Fig. 6.