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METHOD FOR TREATING IMMUNE SYSTEM DYSFUNCTIONS
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- (56) Prior Art Documents
US 4801614
US 4537878
US 4757049
- (57) Claim

1. A method for stimulating the immune system of a patient whose immune system is impaired by chronic use of a narcotic analgesic comprising:

administering an amount of an opioid peptide having at least the amino acid sequence TYR-GLY-GLY-PHE-LEU-ARG-ARG-AA⁸-AA⁹-AA¹⁰-(AA¹¹)_w, wherein AA⁸ is TYR, ILE, LEU or LYS, AA⁹ is ARG or PRO, AA¹⁰ is PRO or LYS, AA¹¹ is LYS, LYS-LEU or LYS-LEU-LYS, w is 0 or 1, and said opioid peptide is in acid or amidated form, the amount administered being therapeutically effective to stimulate bone marrow cells and T-cell mediated components of the immune system of the patient when said immune system is impaired.

2. The method as in claim 1 wherein the opioid peptide is dynorphin (1-17) amide, dynorphin (1-13) amide or dynorphin (1-10) amide.

9. A method for stimulating the immune system of a patient whose immune system is impaired by use of a narcotic analgesic or by radiation comprising:

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administering one or more doses of a dynorphin amide, the doses having at least about 10 mg dynorphin amide per kg body weight per dose.

10. A method for suppressing the immune system of a patient comprising:

administering an amount of an opioid peptide having at least the amino acid sequence TYR-GLY-GLY-PHE-LEU-ARG-ARG-AA⁸-AA⁹-AA¹⁰-(AA¹¹)_w, wherein AA⁸ is TYR, ILE, LEU or LYS, AA⁹ is ARG or PRO, AA¹⁰ is PRO or LYS, AA¹¹ is LYS, LYS-LEU or LYS-LEU-LYS, w is 0 or 1, and said opioid peptide is in acid form, the amount administered being therapeutically effective to suppress the immune system of the patient when said immune system is stimulated, the amount administered being greater than about 500 µg/body weight per dose.

11. The method as in claim 10 wherein the patient's immune system is stimulated by an autoimmune disease or by a transplant.

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(51) International Patent Classification ⁵ : C07K 7/06, 7/08, 7/10	A1	(11) International Publication Number: WO 92/02547 (43) International Publication Date: 20 February 1992 (20.02.92)
(21) International Application Number: PCT/US91/05518 (22) International Filing Date: 2 August 1991 (02.08.91) (30) Priority data: 562,393 3 August 1990 (03.08.90) US (71)(72) Applicants and Inventors: LEE, Nancy, M. [US/US]; 1830 Funston Avenue, San Francisco, CA 94116 (US). ROY, Sabita [MY/US]; RAMAKRISHNAN, S. [IN/US]; 2240 Laurie Road, North St. Paul, MN 55109 (US). LOH, Horace, H. [US/US]; 54 Mendosa, San Francisco, CA 94116 (US). (74) Agents: SIEBERT, J., Suzanne et al.; Majestic, Parsons, Siebert & Hsue, 4 Embarcadero Center, Suite 1450, San Francisco, CA 94111 (US).		(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i> <i>With amended claims.</i> 660420
(54) Title: METHOD FOR TREATING IMMUNE SYSTEM DYSFUNCTIONS (57) Abstract A method is provided for the use of dynorphin in either stimulating or suppressing the immune system of a patient. Where the immune system is impaired, such as from chronic use of a narcotic analgesic (e.g. morphine) or during chemo-radiation therapy, then the method is practiced to stimulate the immune system.		

METHOD FOR TREATING IMMUNE SYSTEM DYSFUNCTIONS

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Field of the Invention:

The present invention generally relates to a method of treating immune system dysfunctions with an opioid peptide, and more particularly to the use of dynorphin in either stimulating the immune system of a patient whose immune system is impaired or by suppressing the immune system of a patient whose immune system is hyperactivated.

Background of the Invention:

Under normal conditions the immune system is strictly regulated from aberrant activation or suppression. Any change in the regulatory mechanisms results in pathological conditions.

For example, virus infections such as HIV lead to the specific elimination of CD4 positive T helper cell population. In the absence of effective immune surveillance, immuno suppressed individuals show increased incidents of EBV associated lymphomas and other diseases associated with secondary infection. For example, bone marrow transplant recipients, whose immune systems have been affected by chemo-radiation therapy, incur a risk of developing lymphomas. Problems associated with the immune system have also been noticed in drug addicts, who comprise a subpopulation vulnerable

to infection by the AIDS virus. These all appear to involve aberrant immune system suppression.

Examples of aberrant immune system activation appear to include the autoimmune diseases, such as
5 rheumatoid arthritis, myasthenia gravis, and the like. Present drug therapies for aberrant immune system activation, such the widely used immuno suppressive compound Cyclosporin A, have restriction on long term use due to side effects.

10 Investigators have recently begun attempts to link immuno regulation to neural opioid systems. It has become increasingly clear there are a number of opioid effects on cells of the immune system, but the mechanisms remain obscure. The authors of a recent
15 review have concluded that the significance of opioids in immune system function remain a matter for speculation. Sibinga and Goldstein, *Ann. Rev. Immunol.*, 6, pp. 219-249 (1988).

The endogenous opioids exist in multiple forms
20 in the central nervous system and include the dynorphins, a series of peptides derived from the precursor prodynorphin (proenkephalin B). Unlike either the enkephalins or the endorphins, many of the dynorphins interact with high affinity with all three
25 major opioid receptor types (μ , δ , and κ). The dynorphins are also nearly unique among endogenous opioids in that they are not analgesic in the brain, although they may be in the spinal cord.

Smith and Lee have recently reviewed the
30 pharmacology of dynorphin in *Ann. Rev. Pharmacol. Toxicol.*, 28, pp. 123-140 (1988). They note a growing body of evidence has indicated that endogenous opioids are closely connected with function of the immune system. The reviewers, however, state that dynorphin
35 had not been tested in any of the studies reviewed, except for one study concerning mononuclear cell

chemotaxis. However, the reviewers note that dynorphin has been implicated in tumor formation.

U.S. Patent 4,361,553, issued November 30, 1982, inventors Loh and Lee, set out the sequence of the first thirteen peptides for the naturally occurring dynorphin (containing seventeen amino acids), which had been discovered to have potent agonist properties in guinea pig ileum and mouse vas deferens. This patent describes the discovery that dynorphin, and particularly dynorphin (1-13) has an opposite effect in hosts tolerant to narcotic analgesic than the effect which has been observed in naive animals (an inhibition of morphine or β -endorphin-induced analgesia). Thus, dynorphin (1-13) potentiates the analgesic effect in tolerant hosts. Dynorphin was found useful in conjunction with a narcotic analgesic in order to reduce the amount of narcotic analgesic administered per dose.

U.S. Patent 4,462,941, issued July 31, 1984, inventors Lee et al., describes dynorphin amide analogs having the sequence



wherein AA^8 is isoleucine, leucine, or lysine, AA^9 is arginine or proline, AA^{10} is proline and a carbonyl carbon at the AA^{10} terminus is amidated. These dynorphin (1-10) amide analogs do not have significant analgesic activity (unless given in huge doses where they tend to produce convulsions), but they differ from dynorphin (1-13) by neither potentiating nor antagonizing morphine in naive animals. In tolerant animals, on the other hand, the dynorphin (1-10) amide analogs appear to be a more potent and selective analog than dynorphin (1-13).

U.S. Patent 4,481,191, issued November 6, 1984, inventors Wei et al., describe a method for treating high blood pressure and disturbances of cardiac

function by administering dynorphin-related opioid peptides, such as dynorphin (1-13) and dynorphin (1-10) amide. It appears that endogenous opioid peptides condition the sensitivity of the peripheral nerves to stimuli that affect heart rate and blood pressure. Thus, circulating opioid peptides, under normal conditions, are operating to control the sensitivity of these peripheral sites of the autonomic nervous system to such endogenous substances. Use of dynorphin in treating high blood pressure modifies the autonomic nervous system so as to amplify and maintain the intensity of endogenous opioid peptides. A mode of action may be by increasing the sensitivity of visceral afferent receptors.

U.S. Patent 4,684,624, issued August 4, 1987, inventors Hosobuchi et al., describe the use of dynorphin-related peptides, in the acid or amidated form, to treat patients suffering from cerebral ischemia. The administration of these opioid peptides to patients suffering from acute focal cerebral ischemia has been found useful in prolonging survival, and appears useful in partially reversing neurologic deficits resulting from cerebral ischemia.

Brief Description of the Drawings:

Fig. 1 graphically illustrates the suppression, or inhibition, of macrophage colony forming units by morphine;

Fig. 2 graphically illustrates the reversal of morphine induced inhibition of macrophage colony forming units by dynorphin (1-10) amide;

Fig. 3 graphically illustrates the reversal of morphine induced inhibition of thymocyte proliferation by dynorphin (1-10) amide; and

Fig. 4 graphically illustrates the dose dependent effect of dynorphin on macrophage colony forming units.

Summary of the Invention:

5 The present invention provides a method for stimulating or suppressing the immune system of a patient by administering different amounts of a dynorphin in the acid form or amide form. The dynorphin administered is an opioid peptide and when in acid form
10 is administered in less than about 200 $\mu\text{g/kg}$ body weight per dose to a patient whose immune system is impaired, so that the immune system is stimulated. Where the dose in acid form is greater than about 500 $\mu\text{g/kg}$ body weight to a patient whose immune system is stimulated, such as
15 by a autoimmune disease, then the immune system is suppressed.

A particularly preferred aspect of the inventive method is to stimulate the immune system of a patient whose immune system is impaired, such as by
20 chronic use of a narcotic analgesic or during chemotherapy.

Detail Descriptions of the Preferred Embodiments:

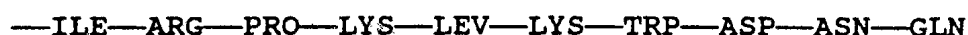
We have discovered that dynorphin and its analogs, when in the acid form, have a biphasic
25 activity. When administered in lower concentrations, such as less than about 200 $\mu\text{g/kg}$ body weight per dose, then the acid form of dynorphin or its analogs stimulates an immune system that is impaired. However, with larger concentrations in acid form, such as doses
30 greater than about 500 $\mu\text{g/kg}$ body weight, the effect is just the opposite and suppresses the immune system.

Suitable compounds for practicing the invention will generally be referred to by the

designation "dynorphin," and have as the first seven amino acids:



The naturally occurring dynorphin has seventeen amino acids, where eight through seventeen are as follows:



Dynorphin analogs that are also suitable in practicing the invention include opioid peptides having at least ten amino acids and optionally with one or more amino acid substitutions (with respect to naturally occurring dynorphin) in the amino acid positions eight through seventeen. Thus, for example, suitable compounds for practicing the inventive method also include within the "dynorphin" designation those having the following structure:



wherein AA^8 is TYR, ILE, LEU, or LYS, AA^9 is ARG or PRO, AA^{10} is PRO or LYS, AA^{11} is LYS, LYS-LEU, or LYS-LEU-LYS, and w is 0 or 1.

Either the acid or the amidated form of dynorphins can be used to practice the immune system stimulating aspect of the invention. Unlike the acid form, the amidated form of dynorphin does not appear to have the biphasic effect. Thus, in the particularly preferred method for stimulating the immune system the amidated form of dynorphin is preferred. Particularly preferred is use of either dynorphin (1-10) amide or (1-13) amide, where the AA^8 is ILE, AA^9 is ARG, and AA^{10} is PRO, and when present, AA^{11} is LYS, AA^{12} is LEU, and AA^{13} is LYS.

We have discovered that chronic treatment with morphine leads to a marked reduction in thymus size, spleen size, inhibition of T-cell maturation, and activation. Using a murine model system with mice
5 (which predictably simulates the conditions of narcotic abuse in humans), the morphine treated animals showed dramatically decreased production of macrophages from the progenitor cells in bone marrow. Therefore, narcotic abuse affects all the major components of the
10 immune system (T-cells, B-cells, and accessory cells) since macrophages play a crucial role in the presentation of antigens to T-cells and are pivotal in the phagocytosis and elimination of infectious agents. Thus, it appears that chronic use of narcotic
15 analgesics, such as morphine, heroin, and others such as those narcotic analgesics clinically used, predisposes the users to a variety of pathological conditions due to immune system suppression.

Turning Fig. 1, the data shows that animals
20 taking chronic morphine do not have new macrophages to resist pathogens. That is, the morphine treatment reduced the proliferative capacity of stem to form macrophages. This reduction in macrophage numbers would predispose the animal to infection because the
25 macrophages are involved in the first line of defense function and in antigen presentation. Thus, mice were implanted with morphine (75 mg) pellets (72 hour pellets) and at different time points, animals were sacrificed. The functional status of the thymocytes and
30 spleen cells were investigated. Mitogenic stimulation assays were used to determine the proliferative capacity (since immunocompetent cells proliferate after activation). The data indicates that the treatment of animals with morphine decreases the ability of T-cells
35 to proliferate. Morphologically, the thymus size was also drastically reduced in morphine treated animals.

As all the T-cells in the body are derived from thymocytes, this reduction in the number of thymocytes and their functional maturation adversely affects the immune system. In parallel studies, bone marrow cells
5 obtained from the same animals were investigated for their ability to produce different lineages of hematopoietic cells under the influence of cytokines. These investigations showed that morphine treatment specifically inhibits macrophage colony formation when
10 the bone marrow cells were stimulated with recombinant M-CSF (which is a growth factor involved in the production and functional maturation of macrophages).

Use of dynorphin, by itself, is not inhibitory to immune cell proliferation, but antagonizes the
15 inhibitory properties of morphine. Thus, in bone marrow cultures *in vitro*, the use of dynorphin improves the macrophage colony formation which had been suppressed by morphine treatment. This is shown by the data of Fig. 2.

20 Similarly, addition of dynorphin to thymocytes prevented the morphine induced suppression of proliferation, as seen by the data of Fig. 3.

The data of Fig. 4 shows that similar effects can be observed in morphine tolerant animals that are
25 injected with either 2 mg or 4 mg/kg body weight of dynorphin. In these experiments animals were implanted with either a 75 mg morphine pellet or a placebo pellet. Each group then received 12 hourly injection of either 2 or 4 mg/kg body weight of dynorphin. As can be seen
30 in Fig. 4 there was a 60% reduction in the bone marrow colony forming units in the morphine tolerant animals injected with saline when compared with the placebo animals injected with saline. However when these tolerate animals were maintained on a dynorphin
35 injection regime the reduction in bone marrow colony formation was significantly increased by about 35%,

suggesting that dynorphin was protecting the inhibitory effects of morphine. Dynorphin when given alone does, however, decrease the colony formation by about 30%.

Preparation of suitable dynorphin compounds for practice of the present invention can be by methods and apparatus known to the art for peptide synthesis. Doses can be administered by intravenous, subcutaneous, or intramuscular injection (I.V., S.C. or I.M) or orally. A preferred range for stimulating the immune system of patient whose immune system is impaired is the administration of between about 10 $\mu\text{g/kg}$ to about 200 $\mu\text{g/kg}$ of body weight, most preferably about 50 $\mu\text{g/kg}$ body weight. Administration can precede the events causing immune system impairment. For example, several hours before chemo-radiation a dose of dynorphin can be administered, preferably followed subsequent to the irradiation by several spaced apart subsequent doses.

Although the experimental animals used in the following experimental descriptions were administered quite high doses (and with three injections per day throughout the morphine administration), humans are believed to be about 20 to 50 times more sensitive to the dynorphin concentrations than mice (although mice are a very good predictive model, except for this difference in dosage sensitivity).

Example 1 more fully describes the protocol from which the data of Fig. 1 was taken. Example 2 describes the protocol from which Fig. 2 taken, and similarly Example 3 for Fig. 3. Example 4 describes dynorphin reversal studies.

EXAMPLE 1

ICR mice from Harlan were used in most studies to see the effect of chronic morphine pellet implanta-

tion on stem cell proliferation. Similar studies were done on Balb C mice from Jackson Labs.

Mice were implanted with two pellets: (1) a 75 mg morphine pellet and a placebo pellet; (2) a 75 mg morphine pellet and a 10 mg naloxone pellet; (3) two placebo pellets; and (4) one placebo pellet and a 10 mg naloxone pellet.

The femurs from both treated and untreated animals were removed aseptically. The bone marrow was flushed through the cut ends by injecting with Iscoves Modified Dulbecco Medium using a 30 gauge needle. One million nucleated cells were resuspended in 0.3% agarose prepared in IMDM containing 30% FCS and plated in a 35 mm petri dish with grids. Colony formation was assessed by treating the bone marrow cells with two different lineage specific growth factors, i.e. recombinant M-CSF (2.5 ng/ml) or mouse GM-CSF (1 ng/ml) for a period of 5 days. The colonies formed were scored using an inverted phase contrast microscope.

20

EXAMPLE 2

ICR mice from Harlan were used in most studies to see the effect of chronic morphine pellet implantation on stem cell proliferation. Similar studies were done on Balb C mice from Jackson Labs.

25

In these studies bone marrow was obtained from untreated naive animals. The bone marrow was assayed from macrophage colonies (CFU-M) and granulocyte-macrophage colonies (CFU-GM) over a range of dynorphin concentration with 100 μ M being the highest concentration and 1 nM being the lowest.

30

The femurs from both treated and untreated animals were removed aseptically. The bone marrow was flushed through the cut ends by injecting with Iscoves Modified Dulbecco Medium using a 30 gauge needle. One

million nucleated cells were resuspended in 0.3% agarose prepared in IMDM containing 30% FCS and plated in a 35 mm petri dish with grids. Colony formation was assessed by treating the bone marrow cells with two different
5 lineage specific growth factors, i.e. recombinant M-CSF (2.5 ng/ml) or mouse GM-CSF (1 ng/ml) for a period of 5 days. The colonies formed were scored using an inverted phase contrast microscope.

EXAMPLE 3

10 ICR mice from Harlan were used in most studies to see the effect of chronic morphine pellet implantation on stem cell proliferation. Similar studies were done on Balb C mice from Jackson Labs.

Thymus was removed aseptically from treated
15 and untreated animals and passed through a nylon mesh to dissociate the cells. The cells were counted using the trypan blue dye exclusion method. The cells were then plated at a cell density of 1×10^6 cells per well in a 96 well plate. Thymocyte proliferation was determined
20 by culturing the cell in the presence of $1 \mu\text{g/ml}$ IL-1. After 48 hours the cells were pulsed for 24 hours with ^3H thymidine ($1 \mu\text{Ci}$) to determine DNA synthesis. At the end of pulse period, cultures were harvested and the cell associated radioactivity was measured.

25

EXAMPLE 4

ICR mice from Harlan were used in most studies to see the effect of chronic morphine pellet implantation on stem cell proliferation. Similar studies were done on Balb C mice from Jackson Labs.

30 Mice were implanted with two pellets: (1) a 75 mg morphine pellet and a placebo pellet; (2) a 75 mg morphine pellet and a 10 mg naloxone pellet; (3) two

placebo pellets; and (4) one placebo pellet and a 10 mg naloxone pellet.

Animals were implanted with either a single morphine pellet (group 1) or a placebo pellet (group 2).
5 Each group was subdivided into 2 subgroups: A and B. Animals in group A received 4 mg/kg body weight Dynorphin (1-13) injections every 12 hours. The animals in group B received saline injections. The animals were maintained on this 12 hourly dynorphin injection regime
10 for 72 hours after pellet implantation and then sacrificed.

The femurs from both treated and untreated animals were removed aseptically. The bone marrow was flushed through the cut ends by injecting with Iscoves
15 Modified Dulbecco Medium using a 30 gauge needle. One million nucleated cells were resuspended in 0.3% agarose prepared in IMDM containing 30% FCS and plated in a 35 mm petri dish with grids. Colony formation was assessed by treating the bone marrow cells with two different
20 lineage specific growth factors, i.e. recombinant M-CSF (2.5 ng/ml) or mouse GM-CSF (1 ng/ml) for a period of 5 days. The colonies formed were scored using an inverted phase contrast microscope.

25 It is to be understood that while the invention has been described above in conjunction with preferred specific embodiments, the description and examples are intended to illustrate and not limit the scope of the invention, which is defined by the scope of
30 the appended claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for stimulating the immune system of a patient whose immune system is impaired by chronic use of a narcotic analgesic comprising:
 - 5 administering an amount of an opioid peptide having at least the amino acid sequence TYR-GLY-GLY-PHE-LEU-ARG-ARG-AA⁸-AA⁹-AA¹⁰-(AA¹¹)_w, wherein AA⁸ is TYR, ILE, LEU or LYS, AA⁹ is ARG or PRO, AA¹⁰ is PRO or LYS, AA¹¹ is LYS, LYS-LEU or LYS-LEU-LYS, w is 0 or 1, and said
10 opioid peptide is in acid or amidated form, the amount administered being therapeutically effective to stimulate bone marrow cells and T-cell mediated components of the immune system of the patient when said immune system is impaired.
- 15 2. The method as in claim 1 wherein the opioid peptide is dynorphin (1-17) amide, dynorphin (1-13) amide or dynorphin (1-10) amide.
3. The method as in claims 1 or 2 wherein an initial dose from about 10 µg/kg patient body weight to
20 about 200 µg/kg patient body weight is administered.
4. The method as in claim 3 wherein the administration is by intravenous, subcutaneous, or intramuscular injection.
5. The method as in claim 3 wherein
25 subsequent doses are administered so long as the patient's immune system is impaired.
6. The method as in claim 1 wherein the patient's immune system is impaired by use of morphine or heroin.



- 7. The method as in claim 1 wherein the opioid peptide is in the amide form and is administered in one or more doses of at least about 10 $\mu\text{g/kg}$ patient body weight per dose.

5 8. The method as in claim 7 wherein the administering is I.V. and is about 50 $\mu\text{g/kg}$ patient body weight per dose.

9. A method for stimulating the immune system of a patient whose immune system is impaired by
10 use of a narcotic analgesic or by radiation comprising:
administering one or more doses of a dynorphin amide, the doses having at least about 10 mg dynorphin amide per kg body weight per dose.

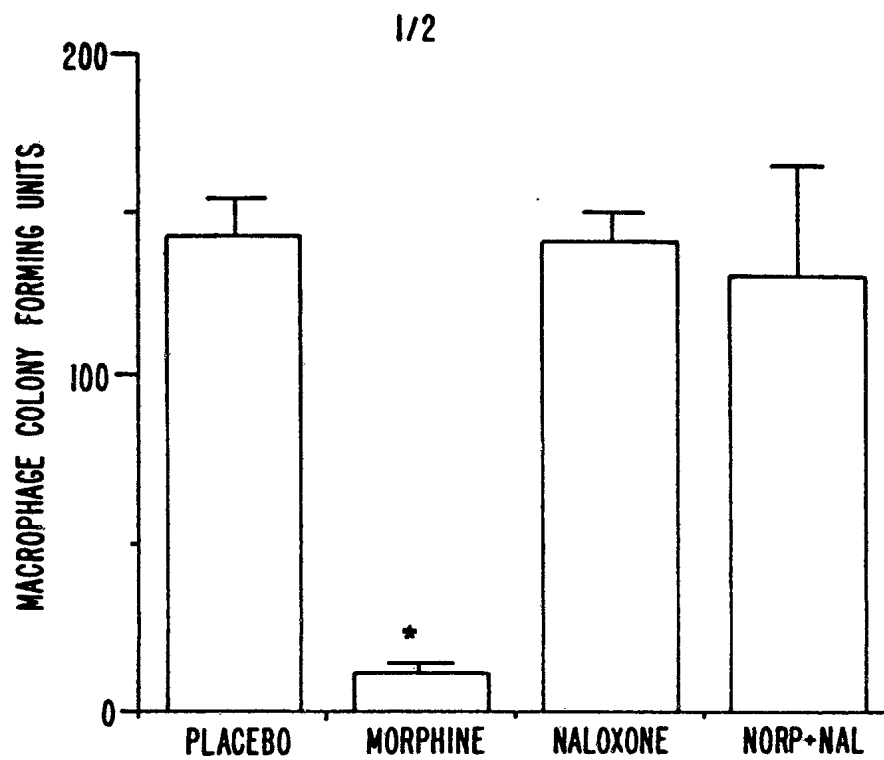
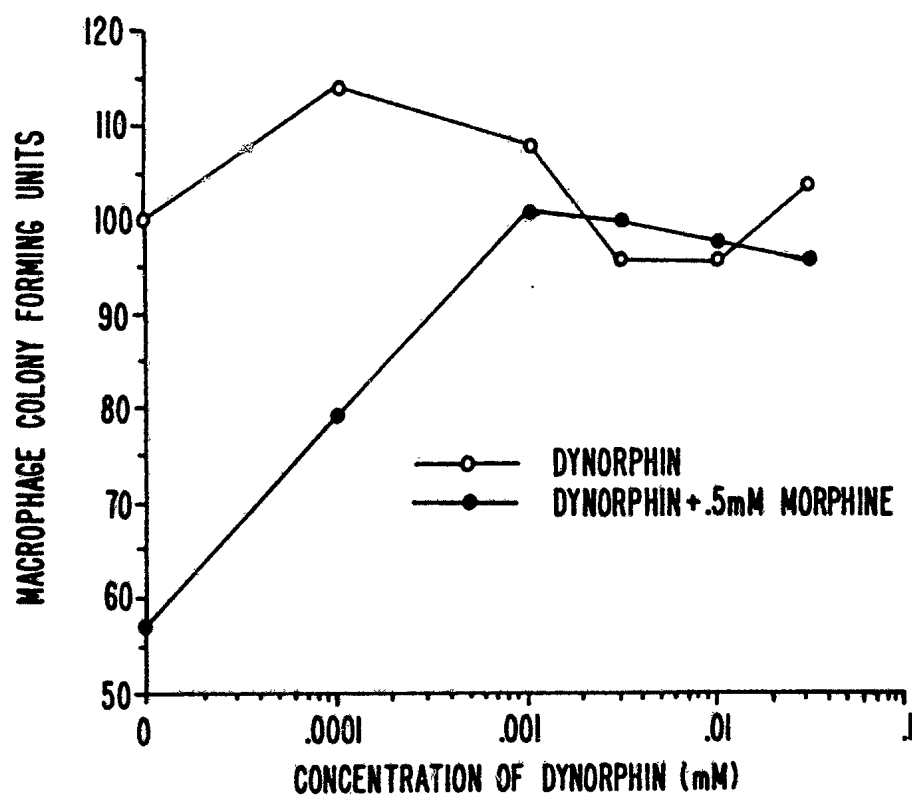
10. A method for suppressing the immune
15 system of a patient comprising:
administering an amount of an opioid peptide having at least the amino acid sequence TYR-GLY-GLY-PHE-LEU-ARG-ARG-AA⁸-AA⁹-AA¹⁰-(AA¹¹)_w, wherein AA⁸ is TYR, ILE, LEU or LYS, AA⁹ is ARG or PRO, AA¹⁰ is PRO or LYS, AA¹¹ is
20 LYS, LYS-LEU or LYS-LEU-LYS, w is 0 or 1, and said opioid peptide is in acid form, the amount administered being therapeutically effective to suppress the immune system of the patient when said immune system is stimulated, the amount administered being greater than
25 about 500 $\mu\text{g/body weight}$ per dose.

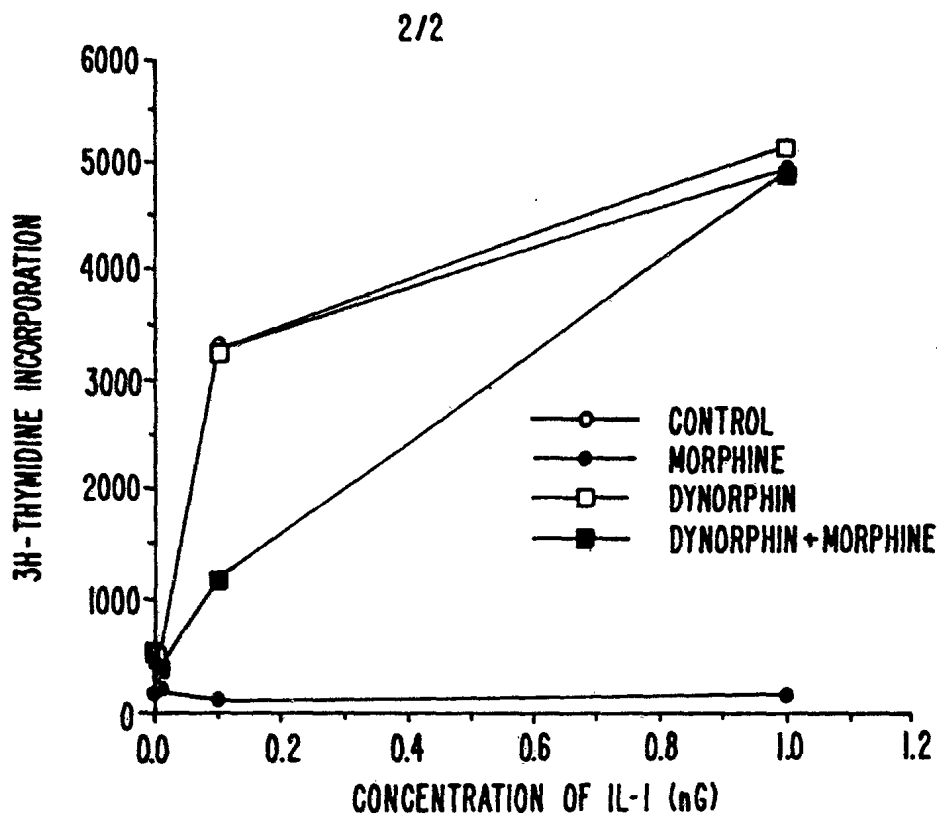
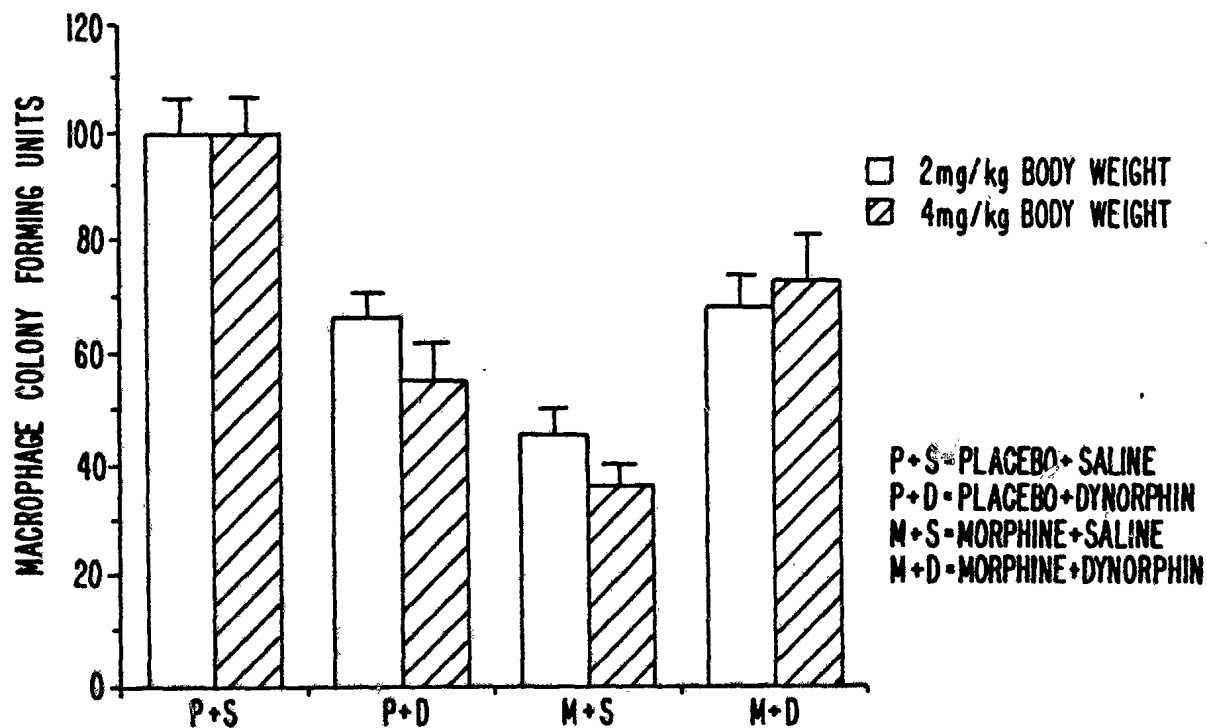
11. The method as in claim 10 wherein the patient's immune system is stimulated by an autoimmune disease or by a transplant.

Dated this 20th day of December 1994.

NANCY M LEE, ROY SABITA, HORACE
LOH and S RAMAKRISHNAN
Patent Attorney for the applicant:
F B RICE & CO



**FIG. 1.****FIG. 2.**

**FIG. 3.****FIG. 4.**

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/05518

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC(5): 007K 7/06, 7/08, 7/10 U.S. Cl.: 514/12,13,14,15,16,17																	
II. FIELDS SEARCHED Minimum Documentation Searched ⁷ <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%;">Classification System</th> <th style="width: 75%;">Classification Symbols</th> </tr> <tr> <td style="text-align: center; vertical-align: middle;">U.S.</td> <td style="text-align: center; vertical-align: middle;">514/12,13,14,15,16,17</td> </tr> </table> Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸			Classification System	Classification Symbols	U.S.	514/12,13,14,15,16,17											
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U.S.	514/12,13,14,15,16,17																
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%;">Category ^a</th> <th style="width: 60%;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="width: 30%;">Relevant to Claim No. ¹³</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">X</td> <td>US,A, 4,537,878 (PLOTNIKOFF) 27 AUGUST 1985, See Abstract and Claims.</td> <td style="text-align: center;">1-14</td> </tr> <tr> <td style="text-align: center;">X</td> <td>US,A, 4,757,049 (PLOTNIKOFF) 12 JULY 1988, See Entire Document.</td> <td style="text-align: center;">1-14</td> </tr> <tr> <td style="text-align: center;">X</td> <td>US,A, 4,801,614 (PLOTNIKOFF) 31 JANUARY 1989, SEE ABSTRACT AND CLAIMS.</td> <td style="text-align: center;">1-14</td> </tr> <tr> <td colspan="2" style="height: 150px;"></td> <td style="text-align: center; vertical-align: middle; font-size: 1.5em;">24333/1</td> </tr> </tbody> </table>			Category ^a	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	X	US,A, 4,537,878 (PLOTNIKOFF) 27 AUGUST 1985, See Abstract and Claims.	1-14	X	US,A, 4,757,049 (PLOTNIKOFF) 12 JULY 1988, See Entire Document.	1-14	X	US,A, 4,801,614 (PLOTNIKOFF) 31 JANUARY 1989, SEE ABSTRACT AND CLAIMS.	1-14			24333/1
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^a Special categories of cited documents: ¹⁰ "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "Δ" document member of the same patent family																	
IV. CERTIFICATION <table style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: top;"> Date of the Actual Completion of the International Search 19 SEPTEMBER 1991 International Searching Authority ISA/US </td> <td style="width: 50%; vertical-align: top;"> Date of Mailing of this International Search Report 28 OCT 1991 Signature of Authorized Officer <i>Lester L. Lee</i> LESTER L. LEE </td> </tr> </table>			Date of the Actual Completion of the International Search 19 SEPTEMBER 1991 International Searching Authority ISA/US	Date of Mailing of this International Search Report 28 OCT 1991 Signature of Authorized Officer <i>Lester L. Lee</i> LESTER L. LEE													
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