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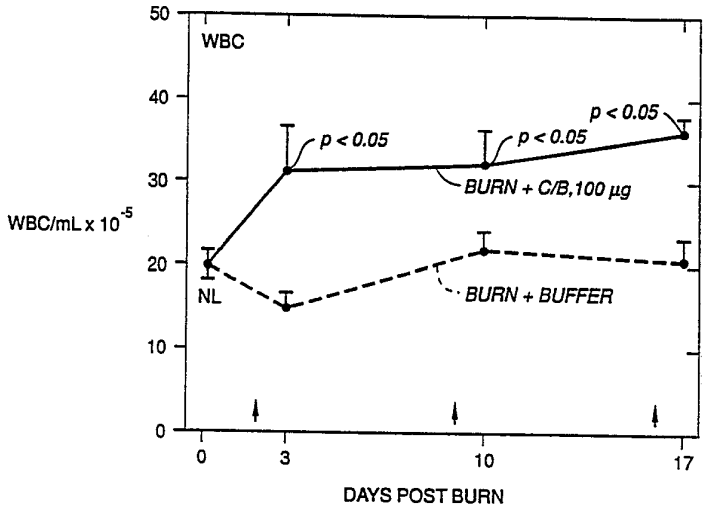
**(54) Title:** METHOD FOR PREVENTING IMMUNE SUPPRESSION IN TRAUMA PATIENTS




**(57) Abstract**

The present invention comprises a method of treating a patient having trauma-induced immune suppression in order to enhance the ability of the patient's immune system to resist opportunistic infection. According to the method a therapeutically effective amount of a non-specific biological response modifier is administered to the patient. Preferably the biological response modifier comprises natural membrane vesicles and ribosomes, in a suspending buffer. Most preferably, the ribosomes and vesicles are both derived from the bacterium Serratia marcescens. The biological response modifier is characterized by absence of substantial toxicity along with product consistency, thus overcoming problems encountered with other immunomodulators. In addition the invention comprises use of a non-specific biological response modifier in the manufacture of a medicament for the treatment of trauma-induced immune suppression. Preferably the non-specific biological response modifier is comprised of natural membrane vesicles and ribosomes in a suspending buffer. Most preferably, the ribosomes and vesicles are both derived from the bacterium Serratia marcescens.

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# METHOD FOR PREVENTING IMMUNE SUPPRESSION IN TRAUMA PATIENTS

This invention relates to the treatment of patients suffering from severe trauma, such as burns. More particularly, the invention relates to the use of a non-specific biological response modifier for improving the ability of traumatized patients to resist opportunistic infections.

## BACKGROUND OF THE INVENTION

Patients suffering from severe burns are particularly prone to infection, not only because of the loss of skin integrity and exposure of necrotic underlying tissue, but also as a result of the profound broad based immunosuppression which occurs within days of the injury. A similar immunosuppression occurs in patients who incur severe trauma other than burns (1).

Both specific and non-specific aspects of immune responses are compromised after such injuries. Pronounced dysfunction occurs in macrophage and neutrophil function and in T-cell mediated responses. Defects in phagocyte function after trauma include antigen presentation and MHC expression (2), chemotaxis (3), superoxide generation (4), and intracellular killing (5). Delayed type hypersensitivity responses (6), allograft rejection (7), and T-dependent antibody responses (8) are reduced after trauma, indicating a generalized T-cell defect. T helper/suppressor ratio is altered in favor of the T suppressor (9) and production of IL-2 is lowered (10) in trauma patients. Natural killer cells (NK), which are an important line of defense against virus infection (11), are not functional in trauma patients, as measured by their ability to kill virally infected target cells (12), although their numbers appear normal (13).

In addition to the functional defects observed in macrophage and neutrophil populations, the numbers of these cells are depleted after severe injury, and examination of bone marrow reveals a lowering in the numbers of stem cells (granulocyte-macrophage colony forming units, GM-CFU) (14) and the presence of humoral factor(s) which inhibit stem cell proliferation. The situation in the trauma patient, therefore, is one of profound disturbance of granulocyte production and function.

In order to prevent opportunistic infections in traumatized patients, various antibiotics have been employed, along with other advances in the care of wounds and burns. Thus, the prognosis for patients suffering from severe trauma has improved. However, many individuals continue to die from overwhelming infections with opportunistic bacteria, viruses or fungi, often because resistant strains of pathogens have rapidly arisen.

Another therapeutic approach for trauma patients is to attempt to reverse the attendant immunosuppression so that the patient's own immune system can counter-attack the invading microorganisms. Since the immunosuppression phenomenon in trauma patients is virtually inevitable and predictable, therapy to mitigate or reverse this phenomenon seems logical. However, because of the rapid and profound collapse of the immune system after severe trauma, it is not possible to predict, with any reasonable degree of certainty, that a particular therapy will be successful in even partially restoring a patient's immunity.

Certain prior art approaches to immunomodulation in trauma therapy have concentrated on the use of purified mediators of the hematopoietic and immune systems - the cytokines and colony stimulating factors. A number of highly purified recombinant human

proteins (IL-2, GM-CSF, IL-3, IL-6) have become available for clinical study.

Although the use of a highly purified single agent with apparently clearly defined biological activities is an appealing prospect, experience in cancer therapy has shown that the administration of unphysiologically large doses of cytokines such as interleukin-2 sets off an as yet ill-defined cascade of other cytokines which can lead to unexpected effects including considerable toxicity. The proliferation, maturation and function of hematopoietic cells is under the control of a complex series of interacting mediators (15). Phase 1 clinical trials of GM-CSF have shown that significant elevations of granulocyte counts can be achieved in cytopenic patients with little toxicity (16), however the cells induced during GM-CSF infusion are functionally defective in at least one respect - the ability to migrate from the blood into tissues (17). This means that while GM-CSF infusion will induce cells capable of dealing with sepsis, infections in tissues such as lung may remain untouched. Thus when used as a single agent, the quantities of GM-CSF required for optimal stimulation of myelopoiesis appear to cause an inappropriate timing of expression of vascular adhesion molecules on granulocytes. The optimal use of recombinant cytokines will almost certainly require combination therapy consisting of the timed administration of multiple factors in a sequence designed to mimic normal hematopoiesis.

Other prior art studies of immunomodulators in trauma treatment have used broad acting "non-specific" agents such as *Corynebacterium parvum* and levamisole. *C. parvum* has been reported to improve the survival of dogs when given as an intravenous infusion 9 days after burn injury (18). However, a subsequent study in guinea pigs given three treatments with *C. parvum* on days 1,3, and 5 after burn injury found no beneficial effects in

preventing artificially induced bacterial peritonitis (19). The second study did find that the anti-helminthic/immunomodulatory drug, levamisole, and the synthetic thymopoetin derivative, thymopentin, were effective in the guinea pig model. Species differences and differing treatment schedules may have accounted for these apparently contradictory results. In humans, a Phase 1 clinical trial of *C. parvum* in burn patients showed an 80% reduction in the bacteremic episodes experienced (20).

These and other broad based or non-specific immunomodulatory agents, although indicating some promise, have drawbacks which could limit their use and/or effectiveness in many trauma patients. Often, bacterially derived agents such as *C. parvum* and muramyl dipeptide are difficult to purify, vary in their potency from lot to lot, have a short shelf-life, and have undesirable side affects. More important, however, is the fact that many of these agents are known to be highly toxic and thus dangerous, especially so in the case of any already severely injured patient.

A new biologic response modifier (BRM), manufactured by Cell Technology, Inc. (CTI) of Boulder, Colorado has been shown to be effective in treating some types of cancers. The absence of substantial toxicity along with product consistency and the ability to lyophilize this BRM make it an attractive product for clinical use. However, it is not apparent or predictable that this material would have a therapeutically significant biological affect with respect to immune compromised trauma patients.

It is an object of the present invention to provide an improved method for treating trauma patients.

Another object of the invention is to provide an improved treatment for trauma patients to enhance resistance to opportunistic infections.

A further object of the invention is to provide an improved non-specific biological response modifier for improving the ability of immune-compromised patients to resist opportunistic infections.

A still further object is to use a biological response modifier which comprises two major particle populations, one of ribosomes and the other of natural membrane vesicles, both of which are endogenous to a microorganism substantially non-pathogenic to humans, which modifier is substantially free of other cellular contaminants, in the manufacture of a medicament for the treatment of trauma-induced immune suppression.

Other objects of the invention will become apparent to those skilled in the art from the following description, taken in connection with the accompanying drawings.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 through 8 illustrate various parameters, as noted on the titles, measured in connection with an experiment using a mouse model.

In the Figures, the following abbreviations are used:

C/B = CTI-BRM,
WBC = White blood count
AGC = Absolute granulocyte count,
AMC = Absolute monocyte count,
ALC = Absolute lymphocyte count,
BM = Bone marrow,
CFU-GM = Colony forming units - granulocyte macrophage
SPL = Spleen

## BRIEF DESCRIPTION OF THE INVENTION

Very generally, in one aspect the invention comprises a method of treating a patient having trauma-induced immune suppression in order to enhance the

ability of the patient's immune system to resist opportunistic infection. A therapeutically effective amount of a non-specific biological response modifier is administered to the patient. The biological response modifier comprises natural membrane vesicles and ribosomes, in a suspending buffer. Preferably, the ribosomes and vesicles are both derived from the bacterium *Serratia marcescens*.

In another aspect the invention comprises use of a non-specific biological response modifier in the manufacture of a medicament for the treatment of trauma-induced immune suppression. Preferably the non-specific biological response modifier will be comprised of natural membrane vesicles and ribosomes in a suspending buffer. Most preferably, the ribosomes and vesicles will both be derived from the bacterium *Serratia marcescens*.

## DEFINITIONS

For the purpose of preciseness, the following terms used in this specification and the appended claims are defined:

"Non-toxic" means within a level of toxicity which is tolerable by the mammalian host receiving biologic response modifier therapy.

"Non-immunogenic" means evoking a sufficiently low immunogenic response, or no response at all, such that undesired, chronic inflammatory and hypersensitivity responses are not elicited, significantly, in the mammalian host.

"Mean diameter" means the mean diameter of MSD Particle Size Distribution Analysis as measured on a BI-90 (Brookhaven Instrument Corp.) particle sizer. The measurement involves an intensity weighting of the size averaging process and is explained more fully in the Operator's Manual for the instrument, Chapter 6, incorporated herein by reference.

"Substantially non-pathogenic in humans" means not or rarely associated with disease in humans of normal health. Since most microorganisms are capable of causing opportunistic infections under the right circumstances, such as in persons whose immune system has been compromised, this definition excludes only those organism which typically cause non-opportunistic infections.

"Tolerable level of endotoxin, cell walls, and cell membrane fragments" means that any such fraction, if present, have low enough level of biologic activity to maintain a non-toxic characteristic as defined herein.

"Immune suppressing response" means an immune response which so attenuates the effect of the desired immune response as to be unacceptable for medical purposes.

"Natural membrane vesicles" means membrane vesicles prepared from membranes which are derived from living or dead nature cells.

## DETAILED DESCRIPTION OF THE INVENTION

The non-specific biological response modifier employed in the method of the invention and its method of manufacture are described in detail in United States Patent 4,971,801 which issued November 20, 1990 from U.S. Serial No. 057,344. A corresponding international application was filed under the provisions of the Patent Cooperation Treaty (PCT Application Number PCT/US87/01397). This international application was published 17 December 1987 under PCT International Publication Number WO87/07503. A full and complete description of the biological response modifier and its method for manufacture is contained in the issued United States patent and the published PCT application. This full and complete description is sufficient to enable a

person having ordinary skill in the art to reproduce the subject material.

For the purposes of this application including the appended claims, the expression "CTI-BRM" shall mean the biological response modifier described in the foregoing application.

CTI-BRM is, at the time of filing this application, undergoing Phase II Clinical Trials for cancer pursuant to regulations of the Food and Drug Administration of the United States of America. Information relating to therapeutically effective amounts of CTI-BRM has been generated in those trials and some of this information is contained in United States Patent 4,971,801 and published PCT Application No. PCT/US87/01397, as well as in other published articles.

For the purposes of this application, a therapeutically effective amount of CTI-BRM is considered to be substantially equivalent to the amount found to be therapeutically effective in connection with cancer, as set out in the above-mentioned United States Patent and published PCT patent application and other publications. However, additional variants of therapeutically effective amounts may be readily determinable by the treating physician through observation, and from the information provided herein. Such are intended to be encompassed within the scope of term "therapeutically effective amount" as used herein and in the appended claims.

Specifically, CTI-BRM comprises natural membrane vesicles and ribosomes in a suspending buffer. The vesicles are comprised of cellular membrane material and are endogenous to a selected organism. The ribosomes are also endogenous to the selected organism. The biologic response modifier is substantially free of intact cells, cell walls, and cell membrane fragments. The selected organism is one which does not evoke an

immune suppressing response, is non-pathogenic in humans, and is one from which membrane vesicles are capable of being formed from cell membrane material and which vesicles are readily endocytosed by the monocyte macrophage cell line. The vesicle population of the CTI-BRM exhibits a mean diameter of at least about 180nm on particle size analysis.

Further description of the CTI-BRM is provided in the aforementioned United States Patent and published PCT application. Such description, and the method of preparation, are set forth with particularity in that application and are incorporated herein by reference. The ability of CTI-BRM to alter the levels of various white blood count and neutrophil levels in cancer patients is described in the aforementioned United States Patent and published PCT application.

Dosage regimens described in the aforementioned United States Patent and published PCT patent application included dosage levels ranging from 0.25 to 10 milligrams administered from 3 to 6 times spaced at 7 day intervals and administered subcutaneously. Toxicity trials indicated no significant toxicity problems with those dosage regimens and further indicated that the product was well tolerated by the human patients. Adjuvant arthritis, granulomas, ulcerations, and similar effects of toxic components are minimized or eliminated by the use of the CTI-BRM.

A preferred source of the material for the CTI-BRM is the organism *Serratia marcescens*. *Serratia marcescens* is a well known organism and many strains are publicly available from a number of sources. For example, some sixty strains are available from the Budapest Treaty approved depository, American Type Culture Collection, Rockville, Maryland, 20852, U.S.A. However, other organisms are suitable as source for the membrane vesicles and ribosomes utilized in the CTI-BRM.

Such microorganisms should be not a member of the microflora of the patient. Moreover, the microorganisms common bacterial antigen must not react or at least must be poorly cross-reactive with organisms making up the normal microflora of the patient. Examples of suitable microorganism sources other than *Serratia marcescens* are *Erwinia chrysanthemi* (pectobacterium) and *Enterobacter aerogenes*.

In manufacturing the CTI-BRM, bacterial cells of a strain of microorganism which is not present in the microflora of the patient to be treated and which has a common bacterial antigen which does not cross react or is poorly cross reactive with organisms making up the normal microflora of the patient to be treated, are cultivated. The cultivated cells are harvested and cell membrane is disassociated with an appropriate detergent. The cellular concentrate is subjected to disruption mechanically at a pressure in excess of 10,000 psi to produce membrane vesicles with a mean diameter not less than 180 nm. The membrane vesicles and free ribosomes are separated from the remaining cellular material in the cellular lysate. The membrane vesicles and free ribosomes are then re-suspended in an appropriate buffer.

CTI-BRM is a powerful immunomodulator: it is rapidly phagocytosed by monocytes/macrophages which then show increased phagocytic, bactericidal and tumoricidal activity (21). Patients injected subcutaneously with CTI-BRM show significant rises in granulocyte counts 24 hours later (22). Co-culture of CTI BRM with human peripheral blood mononuclear cells results in elevation of NK activity (23), increased T-cell mediated cytotoxicity, and augmented lymphocyte and monocyte antibody mediated cytotoxicity (ADCC) (24). The enhancement of these cellular effector functions is most likely a result of a cascade of cytokine release which occurs after CTI-BRM stimulation. Supernatants from

CTI-BRM stimulated human peripheral blood mononuclear cells contain Il-1, IL-2, interferons alpha and gamma, TNF and GM-CSF. Both human and rat CTI-BRM stimulated monocytes produce substantial quantities of a myeloid differentiation factor, as measured by the ability to induce differentiation of the rat C51 chloroleukemia *in vitro* and *in vivo* (25).

This ability of CTI-BRM to stimulate endogenous production of cytokines, inducing proliferation, maturation or enhancement of function of macrophages and lymphocytes, makes it useful for the treatment of post-trauma immunosuppression. Normal mice injected with CTI-BRM via intravenous, intraperitoneal, and subcutaneous routes show an increase in the number of leukocytes in the blood, spleen and peritoneal cavity. Mice suffering a 20% full thickness burn can also respond to CTI-BRM by elevating their blood and splenic leukocyte counts: the majority of these cells are granulocytes and monocytes. Pretreatment of mice given a lethal dose (LD 70-90) of *Listeria* results in significant improvement in survival.

The ability of CTI-BRM to restore the immune systems of traumatized mammals was demonstrated in connection with a mouse model traumatized by burn injuries. The mouse model employed is a well-known one for which results may be readily extrapolated to humans by those skilled in the art. The methods employed were as follows:

1. Burned mouse model.

   Female CF1 mice are dorsally shaved and depilitated with a depilatory cream: 24 hours later the mice are deeply anesthetized with methoxyfluorothane gas, secured in a template and the shaved dorsum exposed to steam for 7 seconds: this results in a 20% full thickness

burn. Immediately postburn, mice are give 1 ml sterile normal saline i.p to counteract shock. In this model, a significant fall in leukocyte count is usually measurable 3 days after burn, and maximal immunosuppression, as measured by the DNCB sensitization assay, occurs 14 days post burn (26).

2. Measurement of leukocyte numbers in blood and spleen.

Blood is collected from the tail or by cardiac puncture under lethal cholorform anesthesia. For spleen cell harvest, mice are killed by $CO_2$ suffocation, the abdomen swabbed with alcohol and overlying abdominal skin cut and pinned back to allow access to the body wall. An incision is made in the body wall and the spleen is removed with sterile forceps. Single cell suspensions of spleen cells are then prepared by known techniques. White cell counts are performed on samples mixed 1:10 with 1% acetic acid, and counted in a hemocytometer chamber. Differentials are performed on standard smear preparations of blood, and on cytocentrifuge preparations of spleen cells, stained with Wright-Geimsa stain (Diff-Quik, Sigma).

3. Measurement of Granulocyte macrophage progenitors in bone marrow and spleen.

$1 \times 10^5$ nucleated tibial bone marrow cells or $1 \times 10^4$ splenocytes per dish are plated in triplicate 35mm petri dishes, and each plate mixed with 1.0ml of 0.3% agar

(Bacto-agar, Difco) in supplemented McCoys medium + 5% FCS. Colony stimulating activity (CSA) is provided by the addition of 50μl of Amicon filtered, dialyzed human urine per dish. Cultures are incubated at 7.5% $CO_2$, 37°C for 7 days, and then the number of colonies containing more than 50 cells enumerated using an inverted microscope. The mean CFU-GM for each cell preparation is calculated and the number of CFU per tibia or spleen determined by multiplying the number of CFU/plate by the quotient of the number of nucleated cells/organ divided by the number of nucleated cells cultured per plate.

4. Measurement of Delayed Type Hypersensitivity.

Postburn days 14 and 15, 25μl of 0.5% DNFB dissolved in 4:1 acetone/olive oil is painted onto the shaved abdomen. Five days later, the ear thickness is each mouse is measured with a caliper and each ear then immediately painted with 20μl of 0.2% DNFB in the acetone/olive oil vehicle. Exactly 24 hours later, ear thickness is again measured and the difference between prechallange and postchallenge thickness calculated.

Two study groups were employed. They were burned mice receiving CTI-BRM, and burned mice receiving NaCl (buffer). The animals received 100mg of CTI-BRM intraperitoneally at postburn days 2, 9, and 16. Twenty-four hours post-inoculation (at days 3, 10, and 17), mice per group were sacrificed and white count,

differential, hematocrit, reticulocyte count, total bone marrow cellularity, total spleen cellularity, and the number of CFU-GM in bone marrow and spleen were assayed in each mouse. The results were not pooled.

Figure 1 shows the mean white count for each experimental group at days 3, 10, and 17 postburn. For reference, Figure 1 shows the mean white count for normal mice whose value was $1.92 \times 10^6$, is indicated by the point NL at the far left. At all three study days, the burned mice receiving CTI-BRM had a significantly higher average white count than burned mice receiving buffer.

In Figure 2, the absolute granulocyte count is illustrated. Burned mice receiving CTI-BRM had marked elevations in absolute granulocyte count compared to burned animals receiving buffer. Again, the point NL at the left indicates a value for normal, untreated mice.

The absolute monocyte counts in the experimental groups are represented in Figure 3. The normal values are shown by the point NL at the far left. CTI-BRM treatment in burned mice resulted in markedly enhanced numbers of circulating monocytes compared to burned mice receiving buffer.

In Figure 4, the absolute lymphocyte counts in the treatment groups are shown. Again, the normal values are shown by the point NL on the far left. Here, however, CTI-BRM had a less significant effect on total lymphocyte counts, the difference being statistically significant only at day 3. However, at day 17, there actually seemed to be a slightly larger number of lymphocytes in burned mice receiving buffer alone.

Total bone marrow cellularity in the tibia of the experimental mice is shown in Figure 5. Again, the normal tibial cellularity is $6.1 \times 10^6$ and is shown by the bar on the left. At day 3, CTI-BRM had no significant effect on total bone marrow cellularity.

CTI-BRM appeared to have no significant effect on bone marrow cellularity.

Figure 6 shows the number of granulocyte macrophage stem cells present in the tibial bone marrow of mice in the experimental groups. CTI-BRM caused a slight increase in CFU-GM tibia at day 3 compared to buffer treated burned mice. However, stem cell numbers fell dramatically in burned mice receiving CTI-BRM or buffer at days 10 and 17. The significance of this awaits further investigation. By day 10, the number of stem cells was approximately equal in both CTI-BRM and buffer-treated burned mice.

At postburn day 17, a marked disparity in spleen sizes was seen between the groups receiving CTI-BRM and those receiving buffer. Splenomegaly was particularly evident when spleens of CTI-BRM treated mice were compared to those of normal, untreated mice.

Splenic cellularity was significantly higher at day 3, 10, and 17 in mice receiving CTI-BRM compared with mice receiving buffer. This is shown in Figure 7.

Figure 8 describes the number of granulocyte macrophage stem cells found in spleens of mice in the study. CTI-BRM appeared to significantly increase the number of splenic stem cells at days 10 and 17 in the burned mice.

When measuring the hematocrits, the burned mice receiving CTI-BRM vs. buffer showed a slight decrease in hematocrit of the CTI-BRM group. However, by the 17th postburn day, the count had returned to normal levels. A similar decrease in hematocrit in normal mice receiving CTI-BRM was seen at days 3, 10 and 17. Again, the hematocrit tended to return to normal levels in the CTI-BRM treated normal mice by day 17.

The decrease in hematocrit can not be explained by a decrease in the production of new red cells because the reticulocyte count (an index of the formation of new red cells) showed that both burned and

normal mice receiving CTI-BRM had higher reticulocyte counts than their control littermates throughout the period of observation. The reason for this is not clear at this time.

In summary, the effects of CTI BRM on postburn myelopoiesis can be stated as follows:

(1) There is significant increase in the total number of circulating white cells, and absolute granulocyte count and absolute monocyte count appear to account for the majority of the increase seen.

(2) Bone marrow cellularity seems to decrease slightly burned mice, and bone marrow stem cells also appear to decrease in number.

(3) Splenic cellularity is increased as are the number of splenic stem cells.

Finally, a mild anemia was associated with CTI-BRM treatment and this was also accompanied by a modest increase in reticulocyte count. The reasons for the anemia and increased reticulocyte count are unclear at this time.

A series of experiments were conducted to evaluate the ability of CTI-BRM to affect the T-cell mediated immune system. To do this, an *in vivo* test was used in which a contact sensitizing antigen, 2, 4-dinitrofluorobenzene, was painted on the shaved and depilitated abdomens of burned mice 16 and 17 days after thermal injury. Five days later, the ear thickness of each mouse was measured with an engineer's caliper and DNFB was painted on the ear surface at a challenging dose. Exactly 24 hours later, the ears were again measured and the degree of ear swelling was used as an index of the competency of the T-cell immune system.

Ear swelling was depressed in burned mice. Burned mice receiving NaCl alone had a significant decrease in ear swelling. The addition of CTI-BRM to

the burn treatment regimen resulted in only modest increases in ear swelling. Normal mice receiving buffer were also mildly immunosuppressed compared to normal controls. However, normal mice receiving CTI-BRM had a correction of any potential immunosuppression to just above normal control levels. These data indicate that administration of CTI-BRM to burned mice has only a modest effect on ear swelling or T-cell mediated immunity in this burned mouse population. Treatment of normal mice appears to have a modest immunomodularity effect as well.

Because some immunomodulators are associated with increase in T-suppressor cell activity, an experiment was conducted using CTI-BRM for just one treatment dose, rather than the two used previously. In this way, it could be determined if a single dose would reduce the induction of suppressor T-cells and be a better treatment regimen for immunosuppressed mice. To accomplish this, mice were burned at day 0 and given a single injection of CTI-BRM at day 9. Abdomens were painted at days 14 and 15 and ear swelling was determined at days 19 and 20. Burn injury again resulted in significant immunosuppression. However, the addition of CTI-BRM had no significant effect on T-cell mediated immunity when looking at the effects of CTI-BRM compared to non-treated controls. A slight increase in ear swelling in the normal mice receiving buffer was modest and not statistically significant. In summary, no advantage appears to be derived from the administration of a single vs. double dose of CTI-BRM.

Administration of the CTI-BRM pursuant to the method of the invention is capable of restoring the immune system in the case of patients suffering from severe trauma. The method of the invention is safe in that it is well tolerated by patients. Restoration of the immune system in such patients restores their

ability to withstand opportunistic infection, thereby greatly enhancing their chances for recovery.

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## SUMMARY

From the foregoing description one of ordinary skill in the art can easily see that the present invention provides a method of treating a patient having trauma-induced immune suppression in order to enhance the ability of the patient's immune system to resist opportunistic infection. According to the method a therapeutically effective amount of a non-specific biological response modifier is administered to the patient. Preferably the biological response modifier comprises natural membrane vesicles and ribosomes, in a suspending buffer. Most preferably, the ribosomes and vesicles are both derived from the bacterium *Serratia marcescens*.

From the foregoing description one of ordinary skill in the art can easily see that the present

invention also teaches use of a non-specific biological response modifier in the manufacture of a medicament for the treatment of trauma-induced immune suppression. Preferably the non-specific biological response modifier will be comprised of natural membrane vesicles and ribosomes in a suspending buffer. Most preferably, the ribosomes and vesicles will both be derived from the bacterium *Serratia marcescens*.

Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying illustrations. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for treating a patient for trauma-induced immune suppression, comprising, administering a therapeutically effective amount of a biological response modifier comprising two major particle populations, one such population being of lesser size particles comprised of ribosomes and the other such population being comprised of natural membrane vesicles, in a suspending buffer, said membrane vesicles and ribosomes being endogenous to a selected microorganism which is substantially non-pathogenic in humans, said biologic response modifier being substantially free of intact cells, and having tolerable levels of endotoxin, cell walls, and cell membrane fragments.

2. The method of Claim 1 wherein said biological response modifier is derived from the microorganism *Serratia marcescens*.

3. The method of Claim 1 wherein said biological response modifier is administered subcutaneously.

4. The method of Claim 3 wherein said biological response modifier is administered at intervals from 2 to 7 days in an amount between about 0.25 mg and 10 mg.

5. The method of Claim 1 wherein said biological response modifier is administered intraperitonealy.

6. Use of a biological response modifier which comprises two major particle populations, one of ribosomes and the other of natural membrane vesicles both of which are endogenous to a microorganism substantially non-pathogenic to man which modifier is substantially free of other cellular contaminants in the manufacture of a medicament for the treatment of trauma-induced immune suppression.

7. Use of a biological response modifier as in Claim 6 wherein said biological response modifier is derived from the microorganism *Serratia marcescens*.

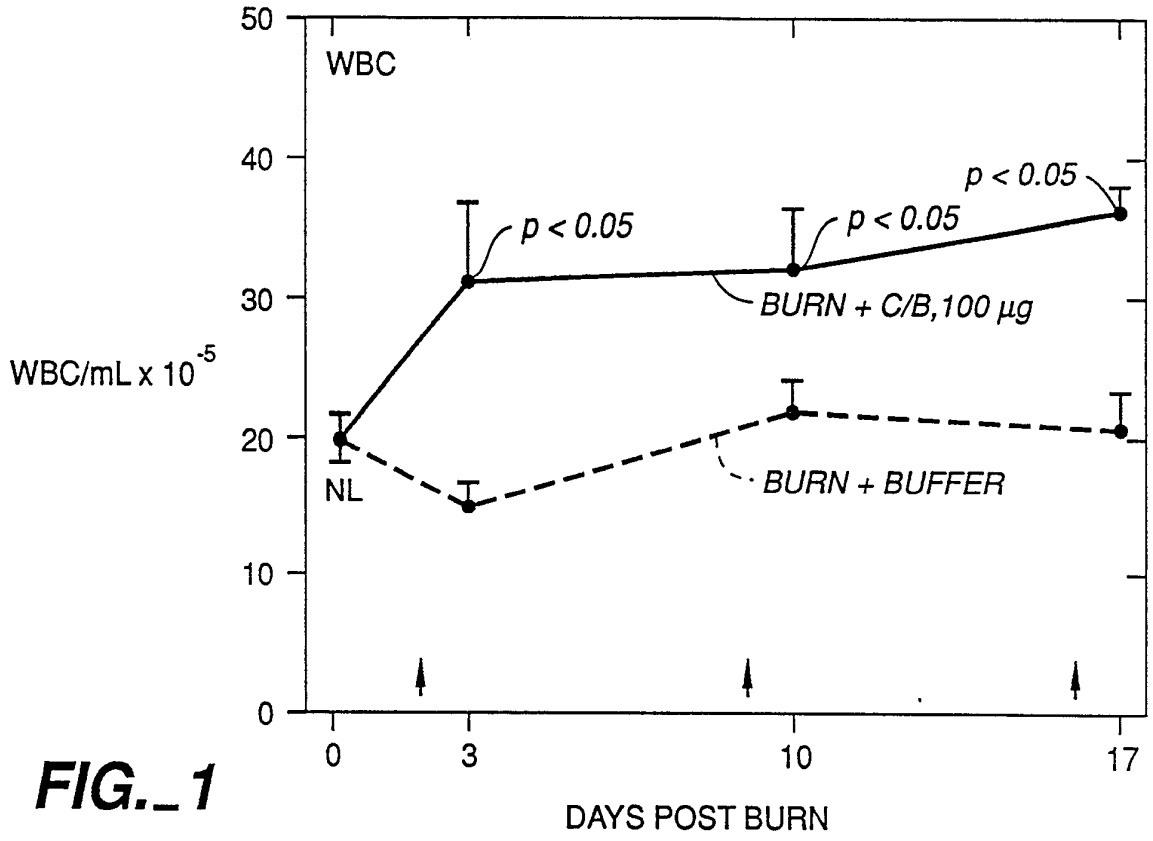
WBC
WBC/mL x $10^{-5}$
50
40
30
20
10
0
p < 0.05
p < 0.05
p < 0.05
BURN + C/B,100 µg
BURN + BUFFER
NL
0
3
10
17
DAYS POST BURN

FIG._1

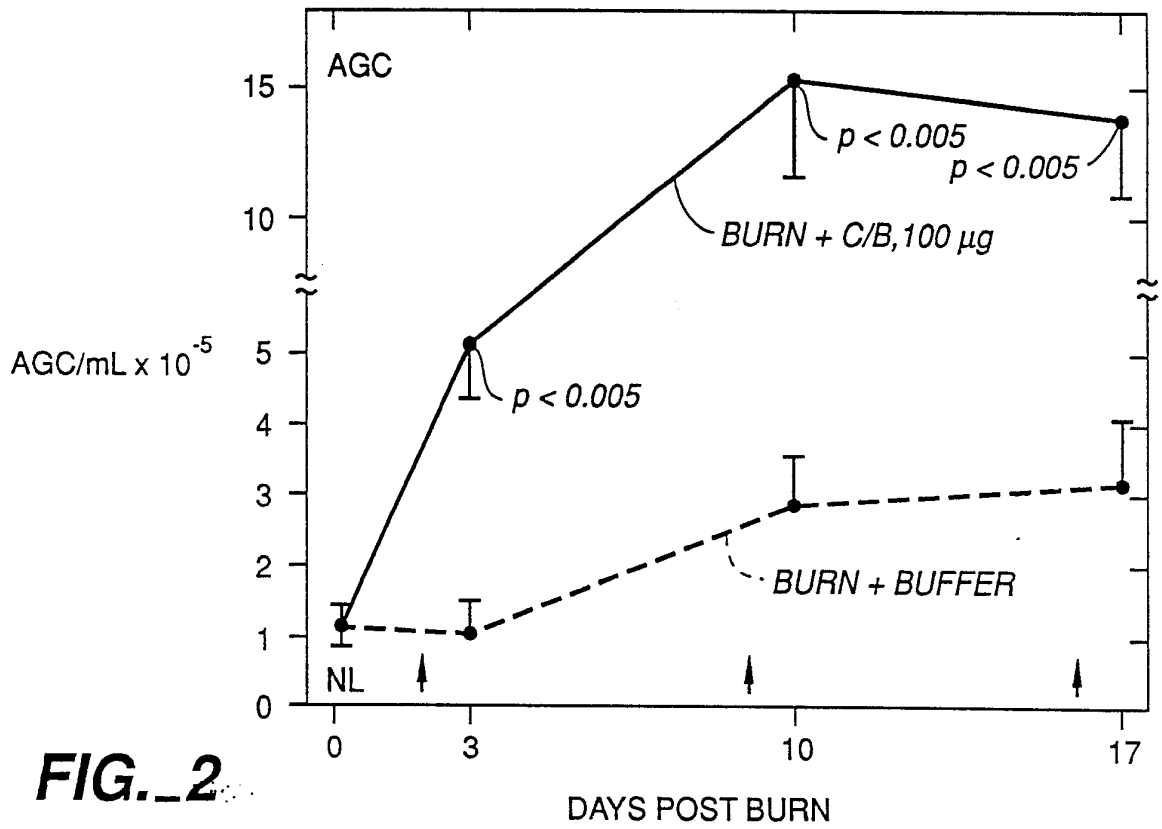
AGC
AGC/mL x $10^{-5}$
15
10
5
4
3
2
1
0
p < 0.005
p < 0.005
BURN + C/B,100 µg
p < 0.005
BURN + BUFFER
NL
0
3
10
17
DAYS POST BURN

FIG._2

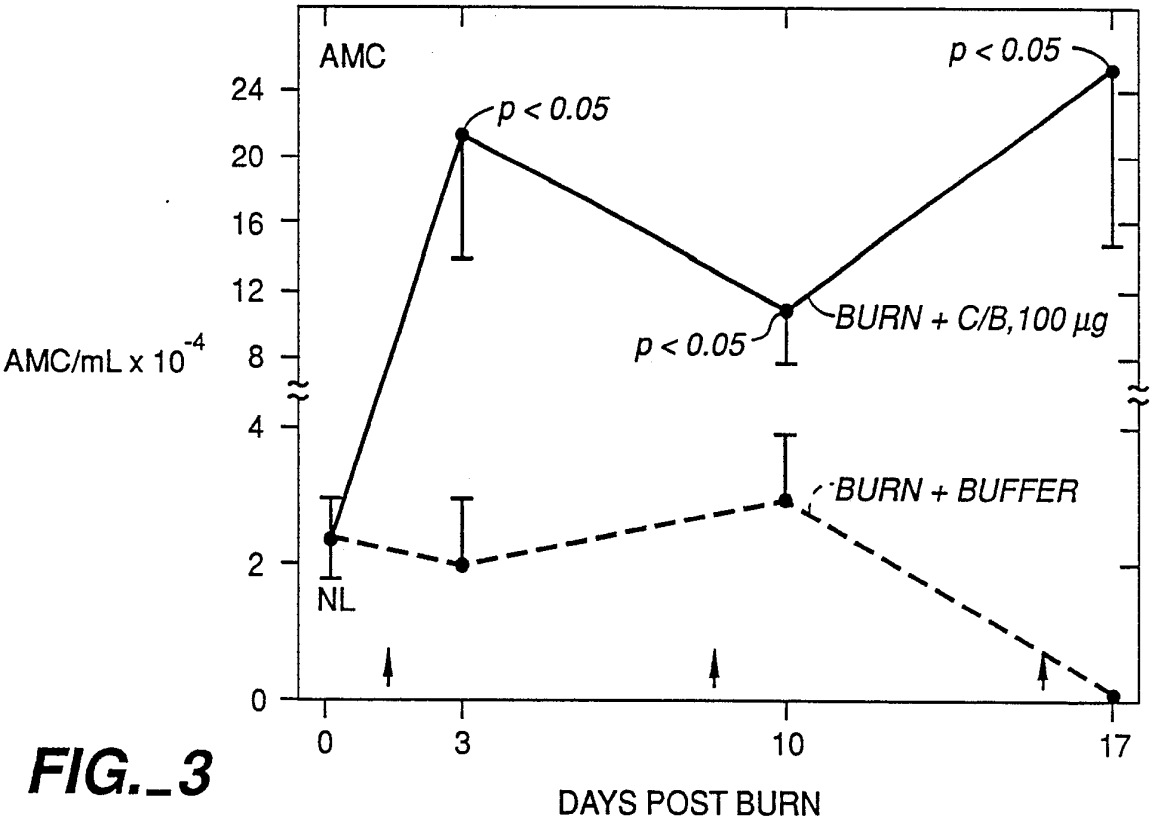
AMC
p < 0.05
p < 0.05
p < 0.05
BURN + C/B,100 µg
BURN + BUFFER
NL
AMC/mL x 10^-4
24
20
16
12
8
4
2
0
0
3
10
17
DAYS POST BURN

FIG._3

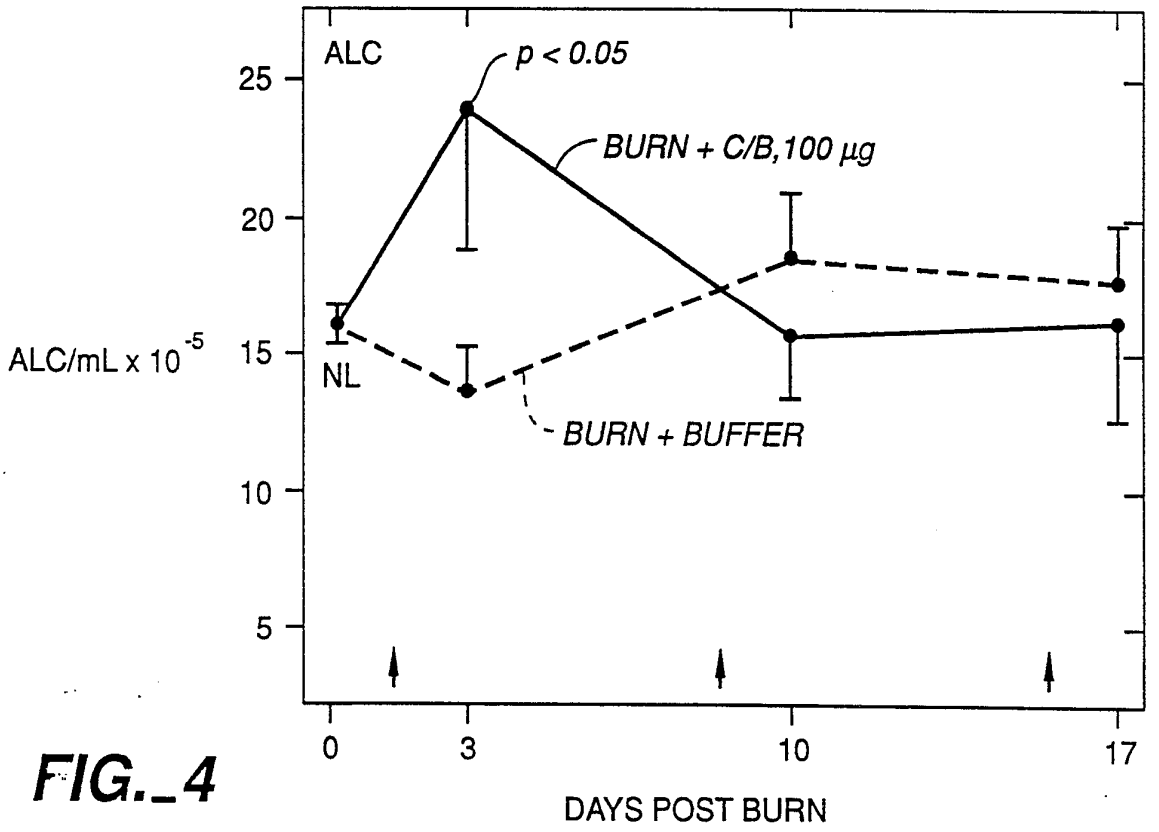
ALC
p < 0.05
BURN + C/B,100 µg
NL
BURN + BUFFER
ALC/mL x 10^-5
25
20
15
10
5
0
3
10
17
DAYS POST BURN

FIG._4

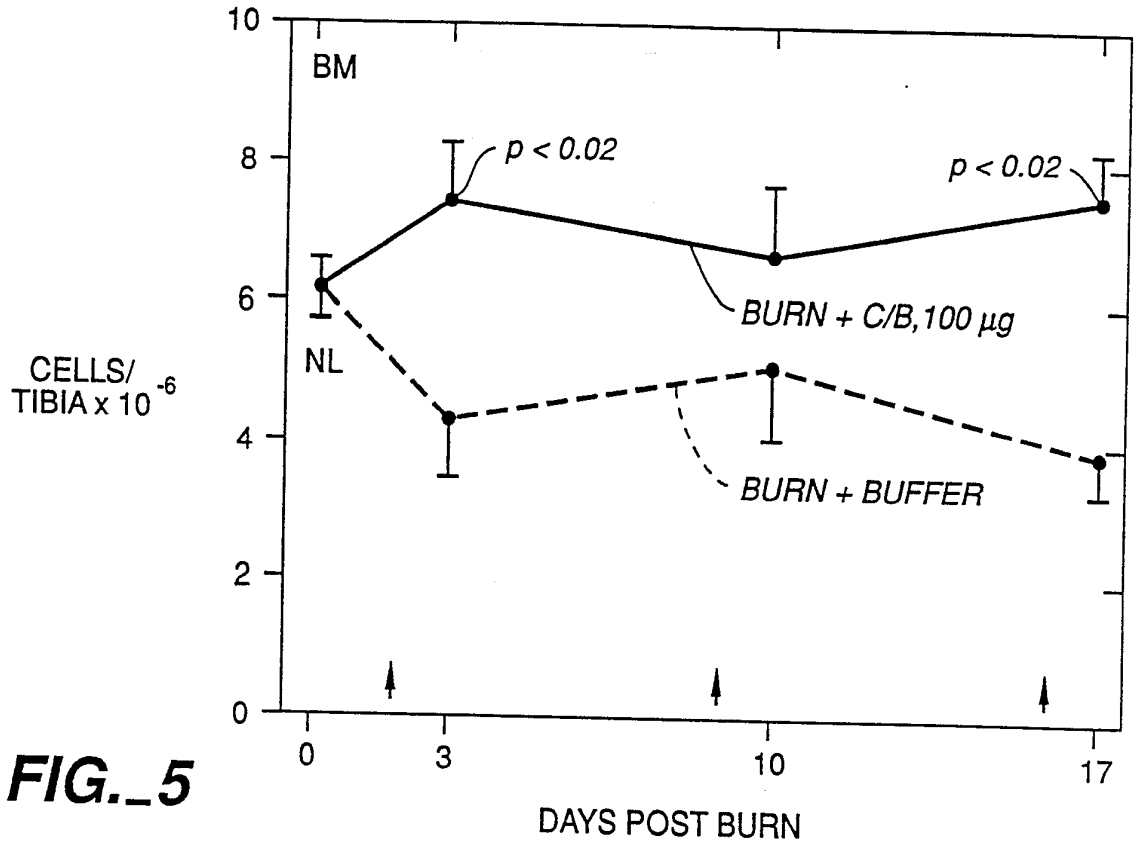
BM
p < 0.02
p < 0.02
BURN + C/B,100 µg
NL
BURN + BUFFER
CELLS/ TIBIA x 10 -6
10
8
6
4
2
0
0
3
10
17
DAYS POST BURN

FIG._5

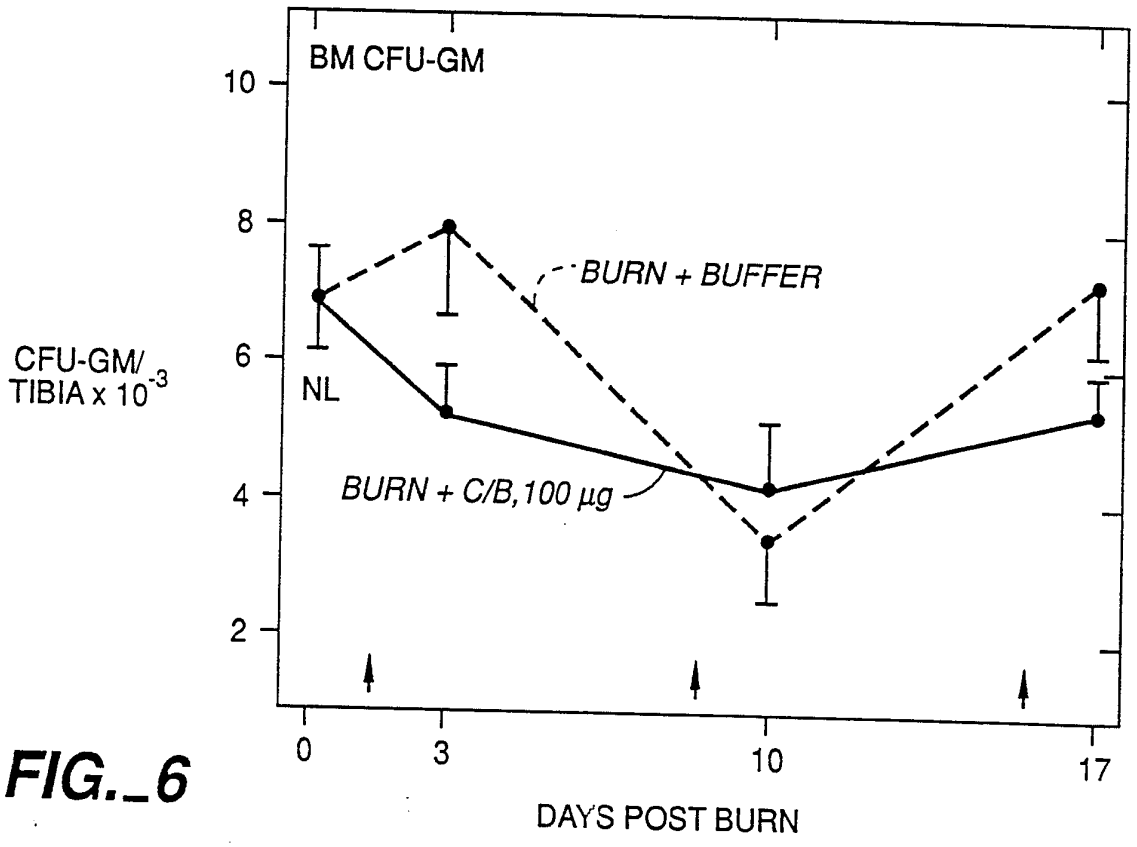
BM CFU-GM
BURN + BUFFER
NL
BURN + C/B,100 µg
CFU-GM/ TIBIA x 10 -3
10
8
6
4
2
0
3
10
17
DAYS POST BURN

FIG._6

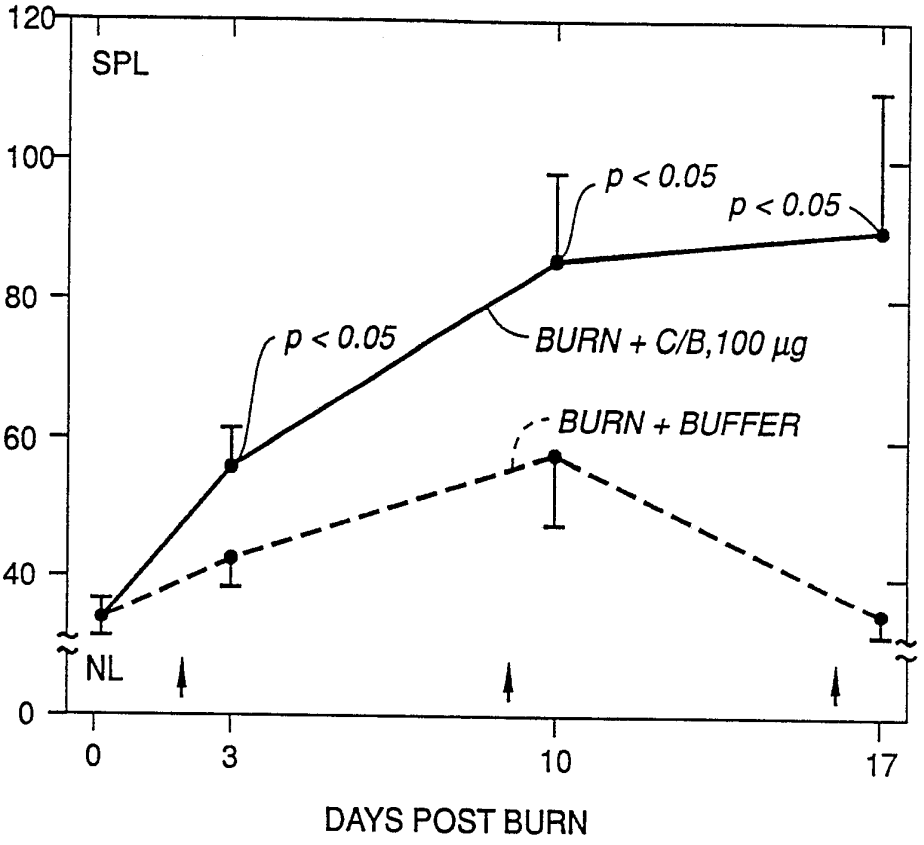
SPL
120
100
80
60
40
0
NL
0
3
10
17
p < 0.05
p < 0.05
p < 0.05
BURN + C/B,100 µg
BURN + BUFFER
CELLS/ SPLEEN x 10 -6
DAYS POST BURN

FIG._7

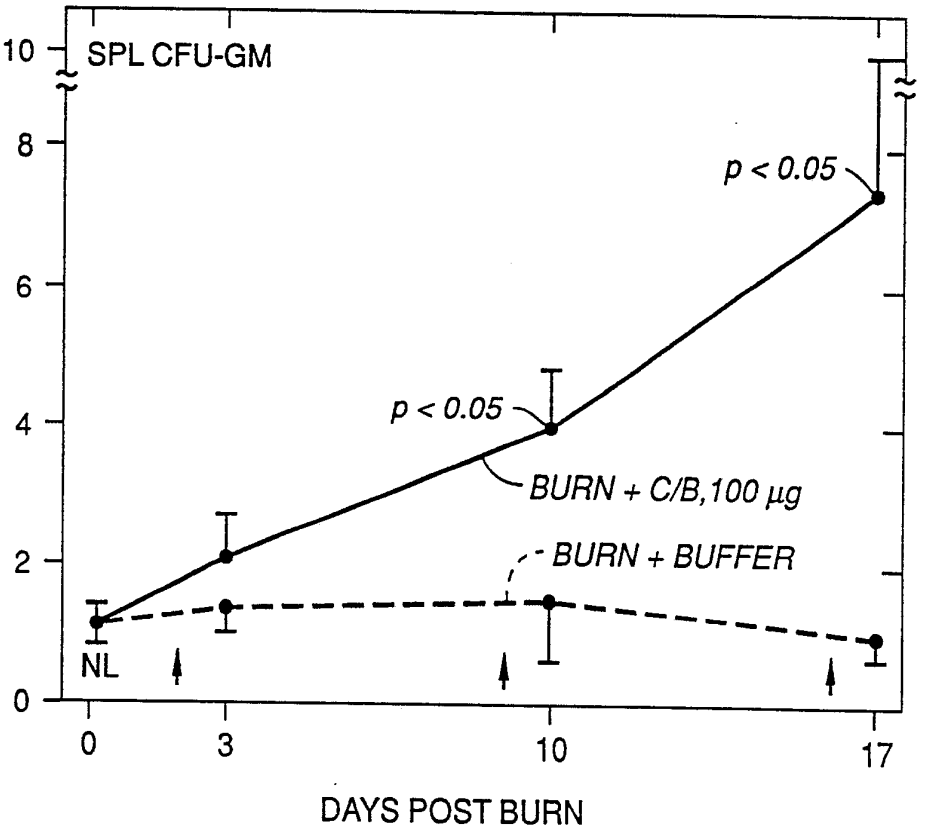
SPL CFU-GM
10
8
6
4
2
0
NL
0
3
10
17
p < 0.05
p < 0.05
BURN + C/B,100 µg
BURN + BUFFER
CFU-GM/ SPL x 10 -3
DAYS POST BURN

FIG._8

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US91/00506

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

INT.Cl. 5 A61K 9/127, 39/02, 39/40, 43/00, 45/05.
U.S.Cl. 424/1.1, 85.2,87,92,450; 428/402.2; 514/885,889.

## II. FIELDS SEARCHED

Minimum Documentation Searched

| Classification System | Classification Symbols |
|---|---|
| U.S.Cl. | 424/1.1, 85.2, 87, 92, 450; 428 |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched

## III. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No. |
|---|---|---|
| X,P | US.A 4,971,801 URBAN 20 November 1990 See Abstract; Col.1, Lines 24-31; Col. 3, Lines 44-50; Col. 4, Lines 11-24; Col. 5, Lines 3-21 and 33-66; Table 2; Col. 11, Lines 25-36; Col.13, Line 21-Col.14, Line 18; Col.15, Lines 10-40; Col.16, Lines 26-68; Col.17, Line 8--Col.18, Line 68; Col.19, Lines 1-51; and Col.20, Line 19--Col.21, Line 49. | 1-7 |
| A | US,A 4,663,161 MANNINO ET AL. 05 May 1987. | 1-7 |
| A | US,A 4,826,687 NEROME ET AL. 02 May 1989. | 1-7 |
| A | US,A 4,871,488 MANNINO ET AL. 03 October 1989. | 1-7 |

* Special categories of cited documents:

"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.

"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 20 March 1991 | 15 APR 1991 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| ISA/US | NGUYEN, [illegible]-HO<br>INTERNATIONAL DIVISION<br>Richard D. Lovering |

Form PCT/ISA/210 (second sheet) (May 1986)