US 20030175277A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2003/0175277 A1**

SHANAHAN-PRENDERGAST (43) **Pub. Date: Sep. 18, 2003**

(54) **THERAPEUTIC FORMULATIONS CONTAINING VENOM OR VENOM ANTI-SERUM EITHER ALONE OR IN COMBINATION FOR THE THERAPEUTIC PROPHYLAXIS AND THERAPY OF NEOPLASMS**

(76) Inventor: **ELIZABETH SHANAHAN-PRENDERGAST**, COUNTY KILDARE (IE)

Correspondence Address:
Kevin Brown
Burr Brown
P.O. Box 7068
Syracuse, NY 13261-7068 (US)

(*) Notice: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

(21) Appl. No.: **09/254,623**

(22) PCT Filed: **Sep. 10, 1997**

(86) PCT No.: **PCT/IB97/01091**

Related U.S. Application Data

(60) Provisional application No. 60/025,179, filed on Sep. 11, 1996.

Publication Classification

(51) **Int. Cl.**[7] **A61K 39/395**; C12P 21/04; A61K 38/46; C12N 9/16

(52) **U.S. Cl.** **424/146.1**; 424/94.6; 435/196; 435/70.21

(57) **ABSTRACT**

The present invention comprises the method of treating host organisms (i.e. human or animal) in need of a drug having anti-neoplastic activity comprising the administration of a therapeutically effective amount of venom anti-serum either alone or preferably in combination with a Phospholipase C inhibitor of non-toxic nature or monoclonal or polyclonal anti-serum to Phospholipase C enzyme or a vaccine containing in whole or in part venom and/or other components of animal, insect or plant origin showing Phosphiolipase A 2 and/or Phospholipase C activity. This patent presents pharmaceutical formulations containing snake and/or insect venoms, or extracts from such venoms which may contain, total or partial, Phospholipase A 2 enzyme activity alone or in combination with animal or plant Phospholipase A 2 with or without Phospholipase C inhibiting compounds or Phospholipase C mono- or polyclonal anti-serum to Phospholipase C enzyme as Phospholipase C mono- or polyclonal anti-serum to Phospholipase C enzyme as therapeutic vaccine candidate for all neoplastic diseases. This patent presents therapeutic pharmaceutical formulations containing anti-serum to snake and/or insect venoms wherein the anti-serum is preferably affinity purified for use in treating neoplastic diseases. This patent presents pharmaceutical formulations containing organic polymer mimic molecules generated to snake and/or insect and/or mammalian and/or plant PLA 2 enzymes or epitopes, or extract from such venoms or synthetic peptides and/or other molecules which may contain, total or partial, Phospholipase A 2 and C enzyme activity.

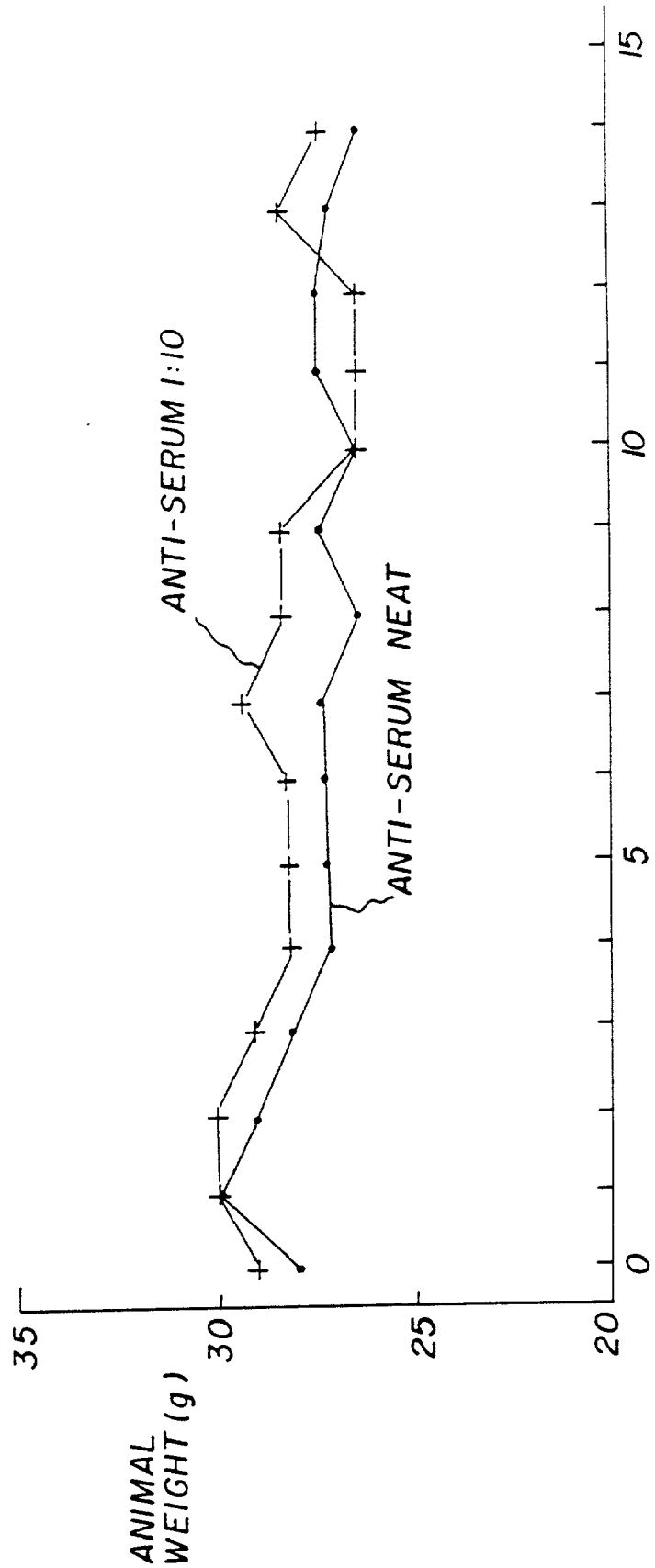
ANIMAL WEIGHT (g)
35
30
25
20
0
5
10
15
TIME (DAYS)
ANTI-SERUM 1:10
ANTI-SERUM NEAT

Fig. 1

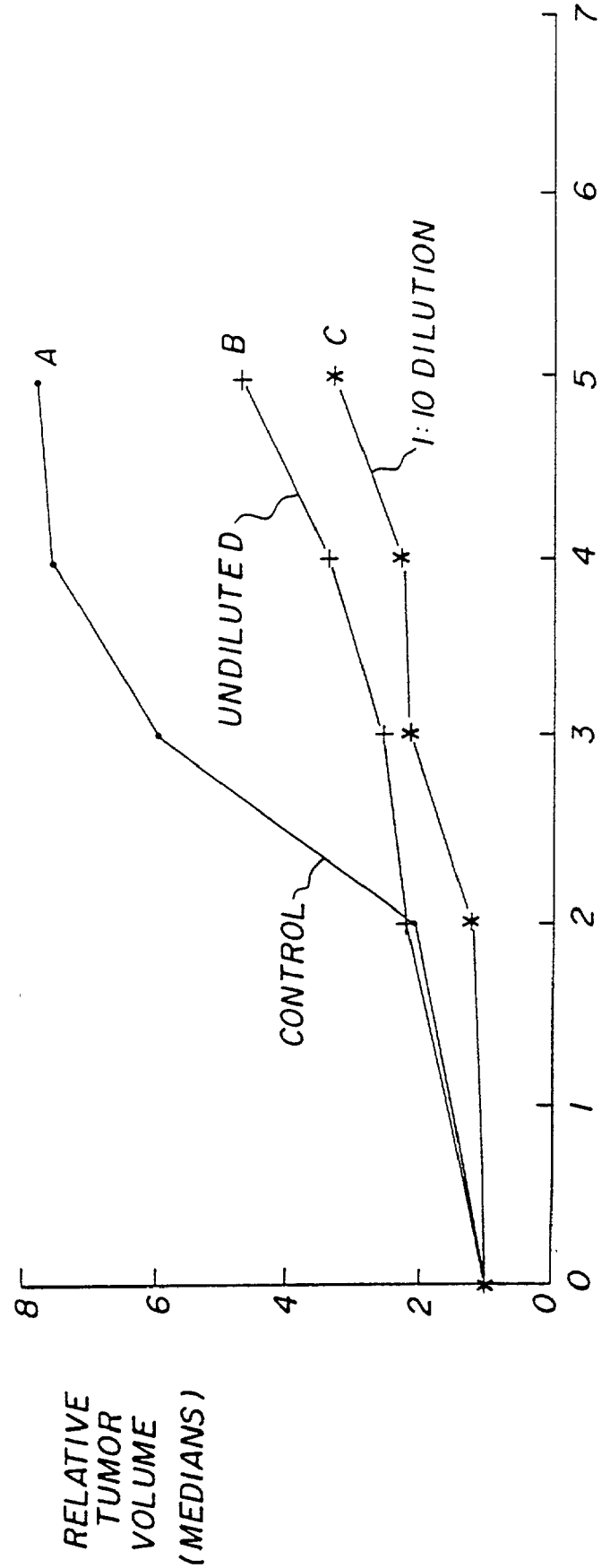

Fig. 2

*Days are measured with day zero taken as day 7 past tumour inoculation.

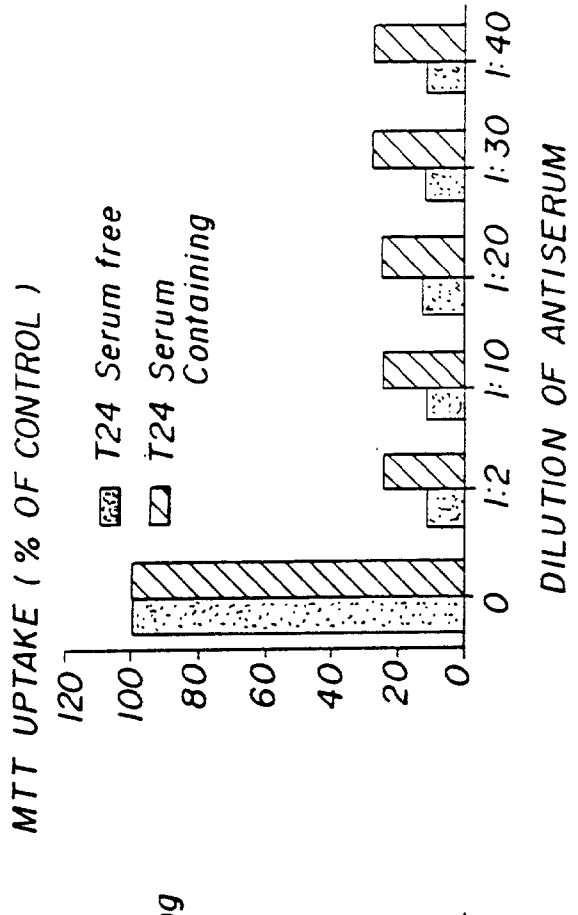
MTT UPTAKE (% OF CONTROL)
120
100
80
60
40
20
0
C170HM2 Serum free
C170HM2 Serum Containing
0
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3A

MTT UPTAKE (% OF CONTROL)
120
100
80
60
40
20
0
T24 Serum free
T24 Serum Containing
0
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3B

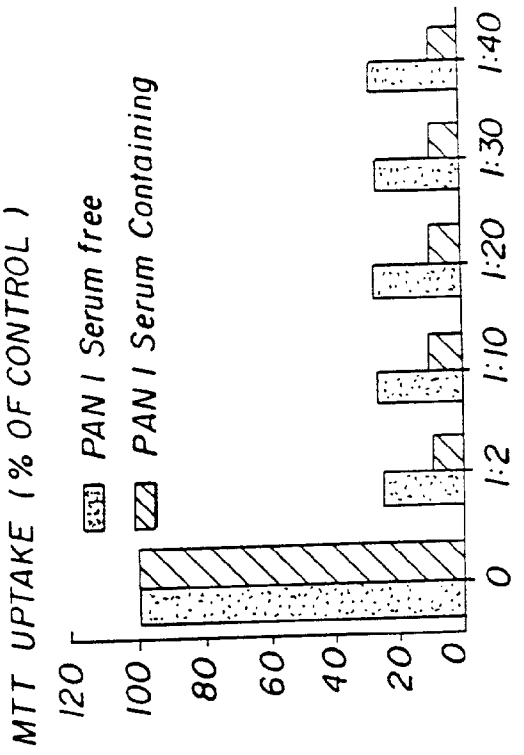
MTT UPTAKE (% OF CONTROL)
120
100
80
60
40
20
0
PAN I Serum free
PAN I Serum Containing
0
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3D

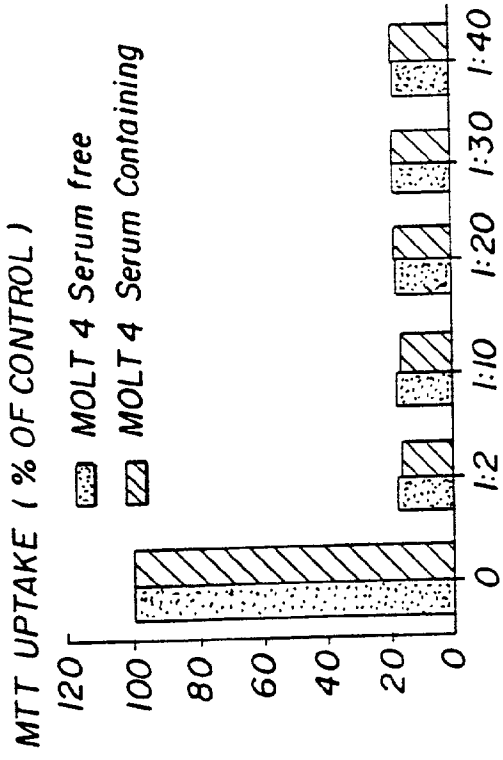
MTT UPTAKE (% OF CONTROL)
120
100
80
60
40
20
0
MOLT 4 Serum free
MOLT 4 Serum Containing
0
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3C

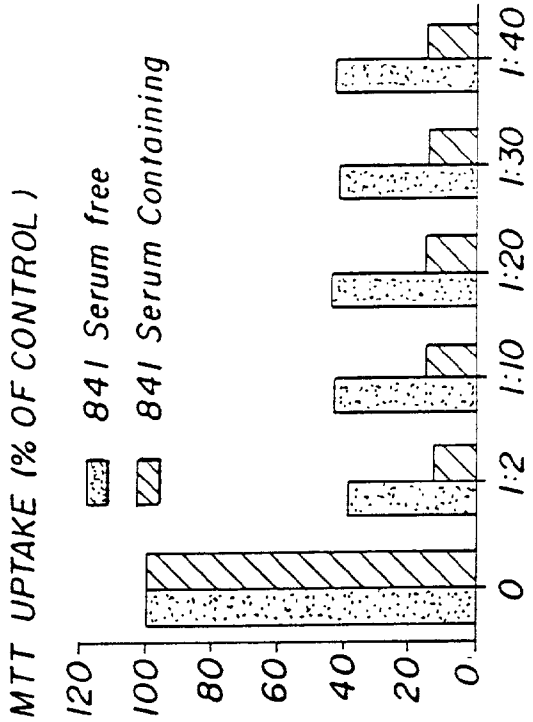
MTT UPTAKE (% OF CONTROL)
841 Serum free
841 Serum Containing
120
100
80
60
40
20
0
0
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3F

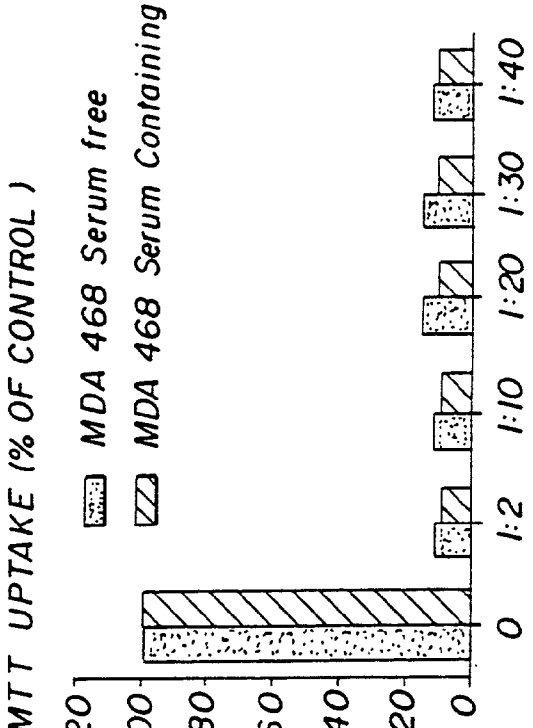
MTT UPTAKE (% OF CONTROL)
MDA 468 Serum free
MDA 468 Serum Containing
120
100
80
60
40
20
0
0
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3E

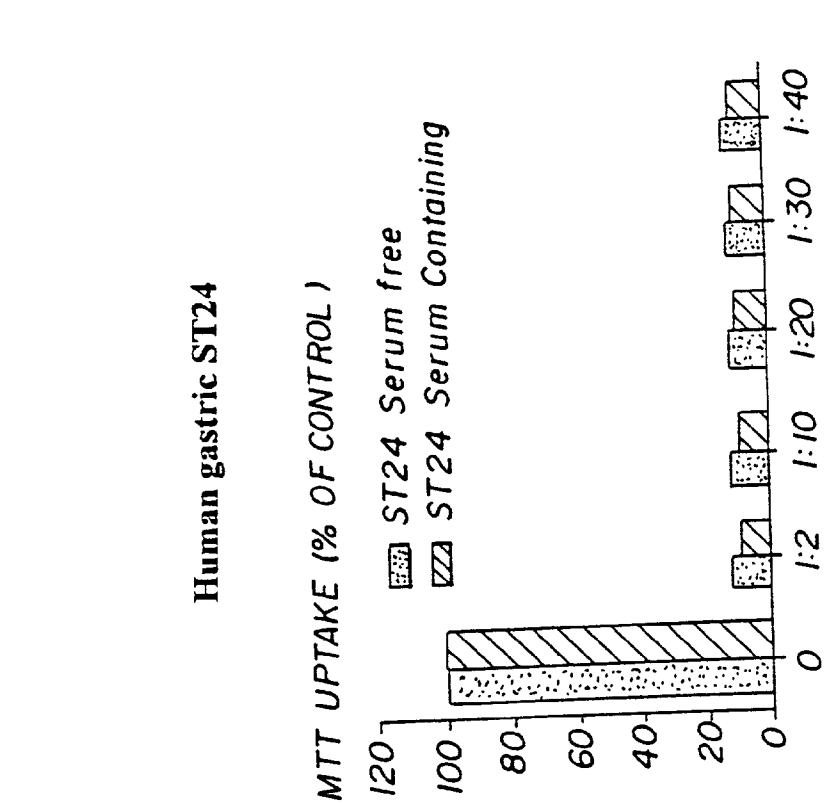
Human gastric ST24
MTT UPTAKE (% OF CONTROL)
ST24 Serum free
ST24 Serum Containing
120
100
80
60
40
20
0
0
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3G

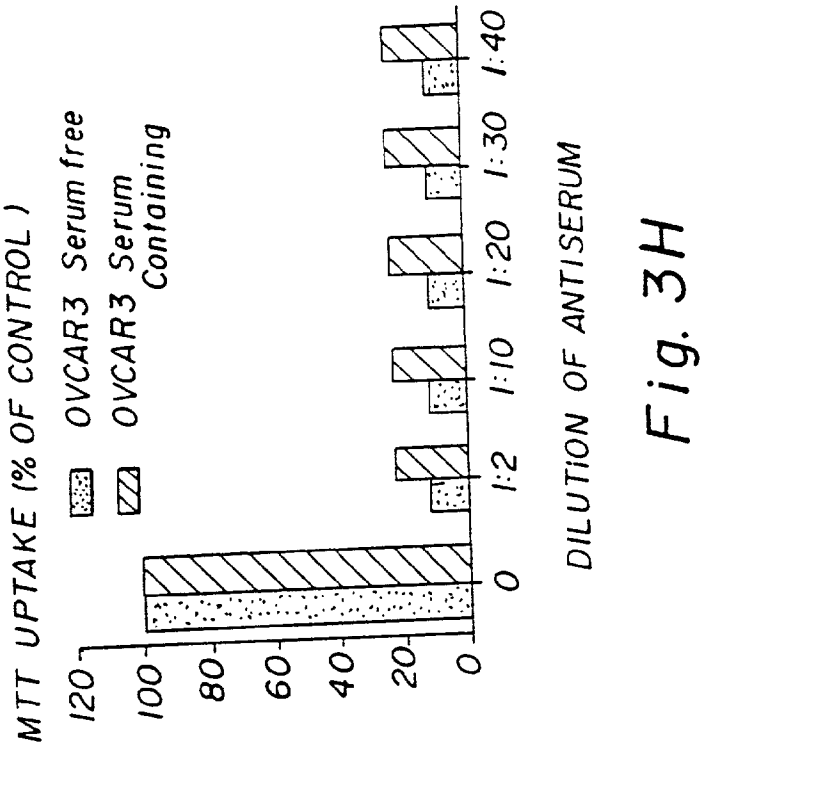
Human Ovarian OVCAR3
MTT UPTAKE (% OF CONTROL)
OVCAR3 Serum free
OVCAR3 Serum Containing
120
100
80
60
40
20
0
0
1:2
1:10
1:20
1:30
1:40
DILUTION OF ANTISERUM

Fig. 3H

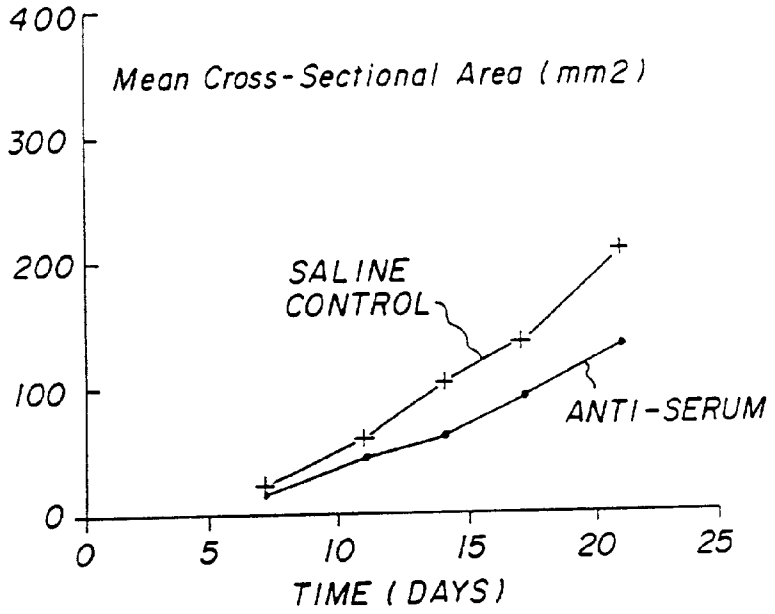
400
300
200
100
0
Mean Cross-Sectional Area (mm2)
SALINE CONTROL
ANTI-SERUM
0
5
10
15
20
25
TIME (DAYS)

Fig. 4

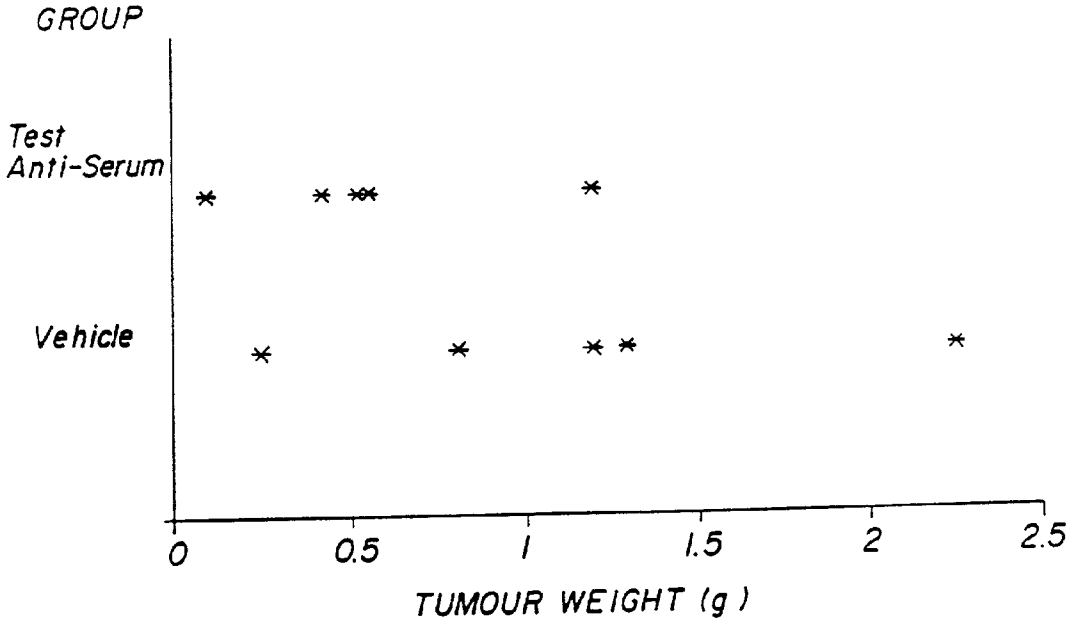
GROUP
Test Anti-Serum
Vehicle
0
0.5
1
1.5
2
2.5
TUMOUR WEIGHT (g)

Fig. 5

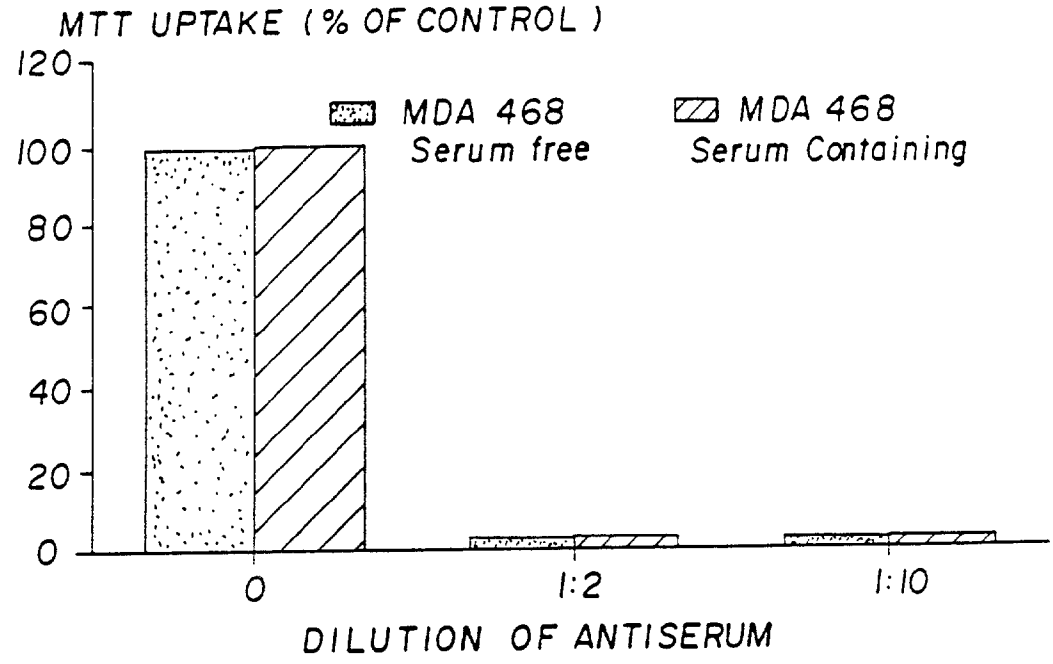
MTT UPTAKE (% OF CONTROL)
120
100
80
60
40
20
0
MDA 468
Serum free
MDA 468
Serum Containing
0
1:2
1:10
DILUTION OF ANTISERUM

Fig. 6A

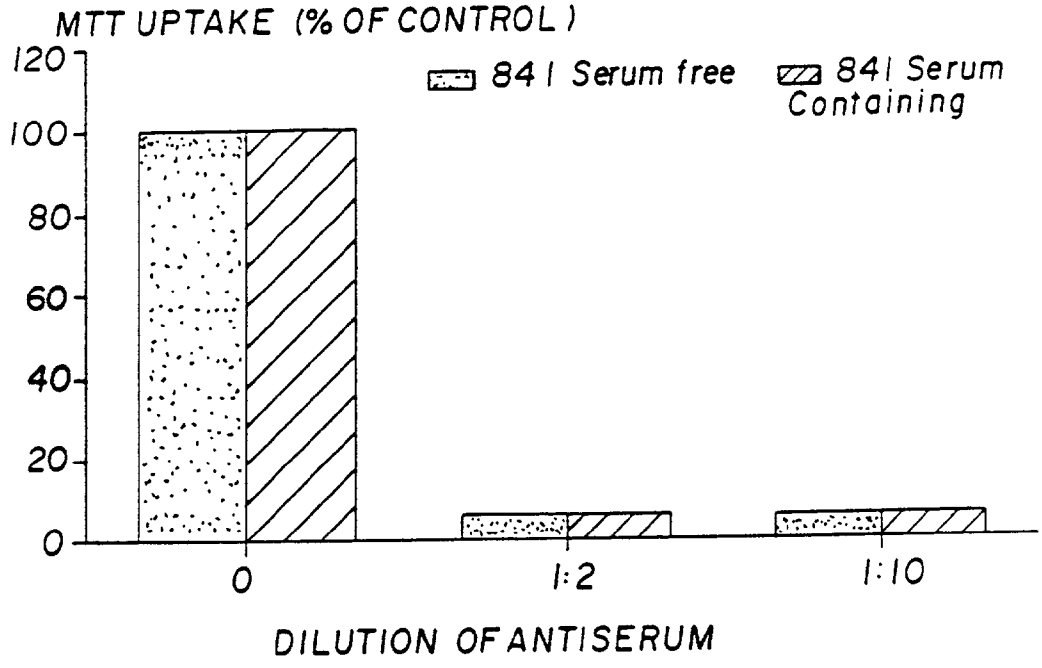
MTT UPTAKE (% OF CONTROL)
120
100
80
60
40
20
0
841 Serum free
841 Serum
Containing
0
1:2
1:10
DILUTION OF ANTISERUM

Fig. 6B

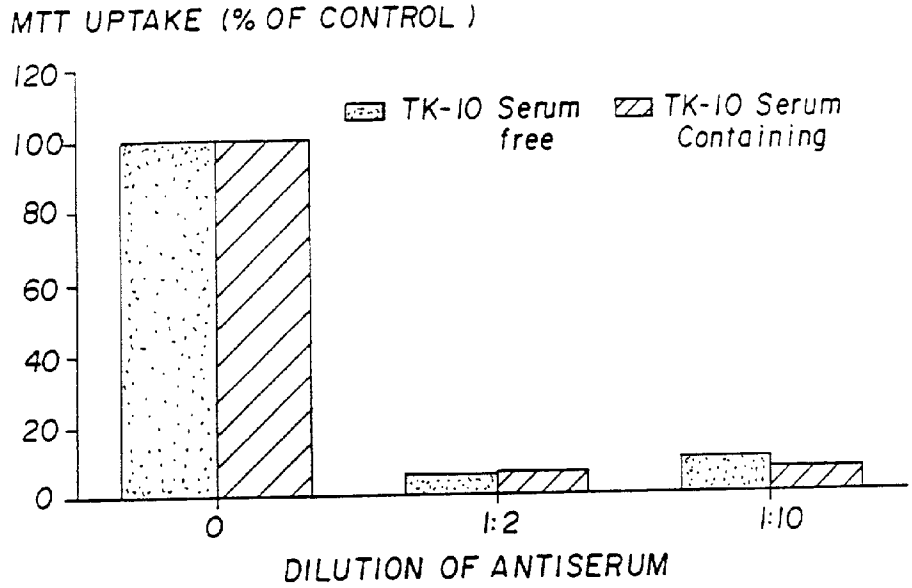
MTT UPTAKE (% OF CONTROL)
120
100
80
60
40
20
0
TK-10 Serum free
TK-10 Serum Containing
0
1:2
1:10
DILUTION OF ANTISERUM

Fig. 6C

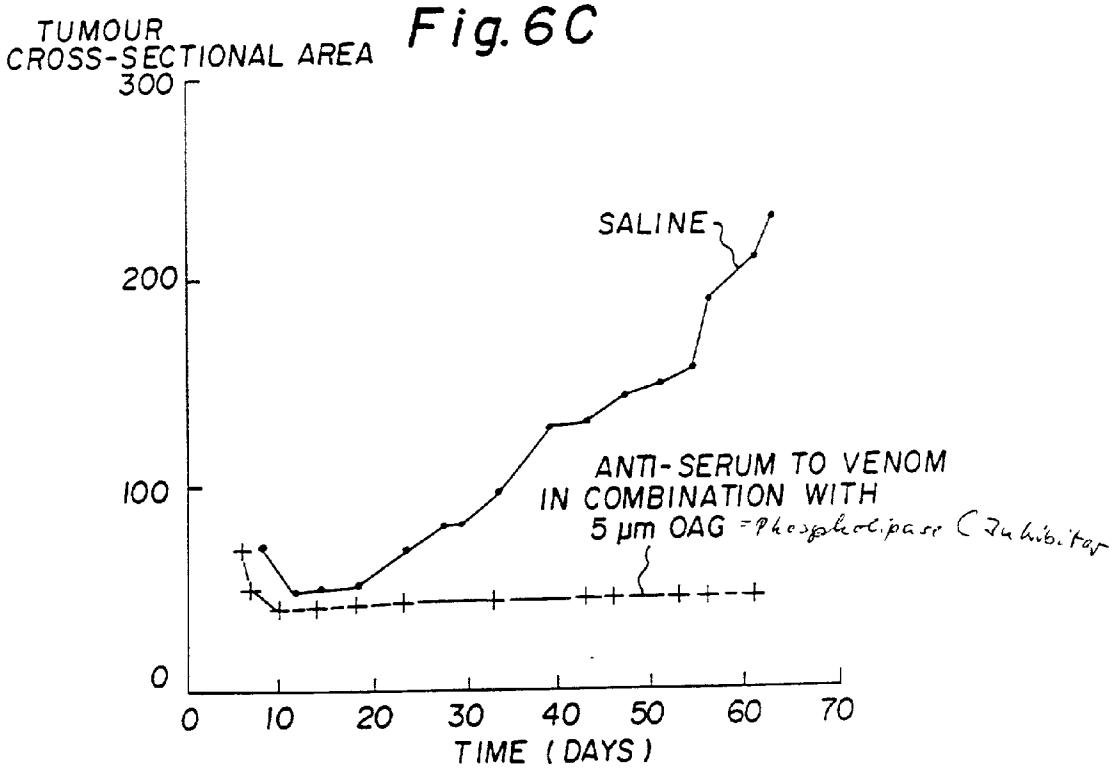
TUMOUR CROSS-SECTIONAL AREA
300
200
100
0
SALINE
ANTI-SERUM TO VENOM IN COMBINATION WITH 5 μm OAG = Phospholipase C Inhibitor
0
10
20
30
40
50
60
70
TIME (DAYS)

Fig. 7

THERAPEUTIC FORMULATIONS CONTAINING VENOM OR VENOM ANTI-SERUM EITHER ALONE OR IN COMBINATION FOR THE THERAPEUTIC PROPHYLAXIS AND THERAPY OF NEOPLASMS

[0001] The present invention comprises the method of treating a host organisms (man or animal) in need of a drug having direct or prophylactic anti-neoplastic activity comprising the administration of a therapeutically effective amount of Phospholipase A_2 targeted venom anti-serum alone or in combination with a known Phospholipase C anti-serum or a Phospholipase C inhibitory compound. A vaccine containing in whole or in part snake or insect venom or mammalian PLA_2 components comprising epitopes demonstrating Phospholipase A_2 activity and/or Phospholipase C enzyme components. This patent presents therapeutic pharmaceutical formulations containing snake and/or insect venoms, or extracts from such venoms which contain, total or partial, phospholipase A_2 enzyme activity or PLA_2 epitopes. This patent presents therapeutic pharmaceutical formulations containing anti-serum to snake and/or insect venoms and/or mammalian PLA_2 enzymes wherein the anti-serum has been preferably affinity purified for use in treating patients suffering from neoplastic disease. This patent presents pharmaceutical formulations containing organic polymer mimic molecules generated to snake and/or insect venoms or the PLA_2 enzyme components thereof and/or PLA_2 enzymes isolated from insect mammalian on plant cells, and/or Phospholipase C enzyme preparation or extract from such venoms which may contain, total or partial, phospholipase A_2 enzyme activity.

[0002] In this patent the affinity purified anti-serum to venoms Phospholipase A_2 (PLA_2) and mammalian or plant PLA_2 are shown to be active anti-proliferative neoplastic agents.

[0003] The present invention comprises the method of treating host organisms (i.e. human or animal) in need of a drug having anti-neoplastic activity comprising the administration of a therapeutically effective amount of venom anti-serum either alone or preferably in combination with a Phospholipase C inhibitor of non-toxic nature or monoclonal or polyclonal anti-serum to Phospholipase C enzyme or a vaccine containing in whole or in part venom and/or other components of animal, insect or plant origin showing Phospholipase A_2 and/or Phospholipase C activity. This patent presents pharmaceutical formulations containing snake and/or insect venoms, or extracts from such venoms which may contain, total or partial. Phospholipase A_2 enzyme activity alone or in combination with animal or plant Phospholipase A_2 with or without Phospholipase C inhibiting compounds or Phospholipase C mono or polyclonal anti-serum to Phospholipase C enzyme as therapeutic vaccine candidate for all neoplastic diseases. This patent presents therapeutic pharmaceutical formulations containing anti-serum to snake and/or insect venoms wherein the anti-serum is preferably affinity purified for use in treating neoplastic diseases. This patent presents pharmaceutical formulations containing organic polymer mimic molecules generated to snake and/or insect and/or mammalian and/or plant PLA_2 enzymes or epitopes, or extract from such venoms or synthetic peptides and/or other molecules which may contain, total or partial. Phospholipase A and C enzyme activity.

[0004] Phospholipase A_2 are lipolytic enzymes that hydrolyze the sn-2-acylester bond in glycerophospholipids. Many forms of PLA_2 exist in nature and have been described and classified into several groups. Types I, II and III PLA_2 are low molecular weight peptides (13-18 kDa) extra-cellular enzymes. including pancreatic and cobra venom PLA_2 (type I), rattle snake and inflammatory PLA_2 (type II) and bee venom type III. Intracellular cytosolic PLA_2 belong to different groups, including the 85 kDa (type IV) and 40-75 kDa enzymes.

[0005] Affinity purified anti-serum to venoms, animal or plant tissue demonstrating the ability to bind PLA_2 enzymes are shown herein below, by way of example, to be active in-vitro and in-vivo anti-proliferative neoplastic agents. Accordingly, these affinity purified antisera either alone or in combination with non-toxic Phospholipase C inhibitor or anti-serum to Phospholipase C are useful in the control of proliferation of neoplastic tissue.

BACKGROUND OF THE INVENTION

[0006] There is evidence to indicate that Phospholipase A_2 (PLA_2) is involved in the pathogenesis of many diseases. Thus local and circulating levels of Phospholipase A_2 enzyme and enzymatic products are elevated during infection, inflammatory diseases, tissue injury and brain dysfunction and is a very early indication of neoplastic development prior to tumour cell mass being evident by conventional methods of scanning tissue tumours.

[0007] Excessive Phospholipase A_2 activity may promote chronic inflammation, allergic reaction, tissue damage and pathophysiological complications These effects may be the result of accumulating Phospholipase A_2 products (lysophospholipids and free fatty acids, e.g. Arachidonic Acid) and destruction of key structural phospholipid membrane components, but are potentated by secondary metabolites, such as eicosanoids and platelet-activating factor. Phospholipase A_2 products or lipid mediators derived therefrom have been implicated in numerous activities that are an integral part of cell activation: chemotaxis, adhesion, degranulation, phagocytosis and aggregation.

[0008] Phospholipase A_2 secreted excessively at local sites may be responsible for tissue damage common to rheumatic disorders, alveolar epithelial injury of lung disease and reperfusion.

[0009] During acute myocardial ischemia, cytosolic Phospholipase A_2 and Phospholipase C activation causes increased intracellular Ca^{2+}. Subsequent accumulation of lysophospholipids and free fatty acids promote damage to sarcolemmal membranes leading to irreversible cell injury and eventually cell death.

[0010] Altered cytosolic Phospholipase A_2 and Phospholipase C activity or defects in their control and regulation is a predisposing factor to causing tumour cell development.

[0011] Prostaglandins and related eicosanoids are important mediators and regulators of both immune and inflammatory responses. Prostaglandin E_2 induces bone resorption and Leukotriene B_4 stimulates vascodilation and chemotaxis. Increased levels of Phospholipase A_2 is noted in Rheumatoid Arthritis (R. A.), osteoarthritis, gout, collagen and vascular diseases Phospholipase A_2 induces non specific airway hyperactivity that is evident in asthma. Phospholi-

pase A_2 is also elevated in peritonitis, septic shock, renal failure, pancreatis, Chrons and Graves Disease.

[0012] The activity of cell-mediated defence systems is stimulated by consecutive formation of interleukin 1β(IL-1β), interleukin-2 (IL-2) and interferon γ (IFN γ). The system is inhibited by interleukin-4 (IL-4) and also by prostaglandin E_2 (PGE_2) and histamine, which are released when the immune system is activated. The inhibition is strong in cancer patients, because PGE_2 is formed in many cancer cells and its formation is stimulated by IL-1β. PGE_2 and histamine are feedback inhibitors of cell mediated immunity.

[0013] PGE_2 is formed from arachidonic acid in monocytes, macrophages, cancer cells and other cells, when aracnidonic acid is released from cellular phospholipids. The formation of PGE_2 is stimulated by several compounds, including histamine, IL-1 (αand β) and Tumour Necrosis Factor α (TNFα). PGE_2 inhibits the formation and receptor expression of IL-2 by increasing the level of cyclic AMP (cAMP) in helper T cells. This concomitantly decreases the formation of IFN γ.

[0014] PGE_2 inhibits the ability of natural killer cells (NK) to bind with tumour cells by increasing cAMP in Natural Killer Cells. This decreases tumour cell killing.

[0015] When the immune system is stimulated to destroy tumour cells. the killing is prevented because IL-1β stimulates PGE_2 formation in tumour cells, which increases cAMP levels in NK cells and prevents the binding of NK and tumour cells.

[0016] The activation of the cell-mediated defence is blocked also because PGE_2 -increases cAMP in helper T cells and inhibits the formation of IL-2 and IFNγ.

[0017] Cytotoxic T cells can also produce PGE_2 thus inhibiting the activity of NK cells.

[0018] A number of human and experimental animal tumours, contain and/or produce large quantities of prostaglandins (PG). Prostaglandins E_2 has been shown to effect significantly cell proliferation in tumour growth and to suppress immune responsiveness.

[0019] Phosphatidylinositol specific phospholipase C is an important enzyme for intracellular signalling. There are at least three major classes of Phosphatidylinositol specific Phospholipase C (PtdInsPLC. PtdInsPLC β, γ, δ). PtdInsPLCs hydrolyse a minor membrane phospholipid, phosphatidylinositol (4, 5) bisphosphate (PtdIns (4,5) P_2) to give the second messengers inositol (1, 4, 5) trisphosphate (Ins (1, 4, 5) P_3), which releases Ca++ from intracellular stores to increase the intracellular free CA++ concentration, and diacylglycerol which activates the Ca++ and phospholipid-dependent protein serine/threonine kinase, protein kinase C. Proteins phosphorylated by protein kinase C include transcription factors. Together, the increase in intracellular free Ca++ concentration and the activation of protein kinase C lead to a series of events that culminate in DNA synthesis and cell proliferation in tumour cells.

[0020] A number of growth factors and mitogens, including platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and bombesin, act through specific receptors to increase Ptd Ins PLC activity in cells. Continued stimulation of Ptd Ins PLC can lead to cell transformation.

[0021] Ptd Ins PLC activity is found to be increased in a number of human tumours. 76% of human breast cancers have detectable Ptd Ins PLC-γimmunoreactive protein compared to only 6% in benign breast tissue,

[0022] Cytosolic Ptd Ins PLC activity is increased up to >4-fold in human non-small cell lung cancer and renal cell cancer compared to normal tissue.

SUMMARY OF THE INVENTION

[0023] The present invention comprises the method of treating mammals including humans in need of a drug to prevent neoplastic tissue growth and spread by the administration of a therapeutically effective amount of venom anti-serum prepared to whole venom or to parts of the venom or components of plant or animal origin which demonstrate PLA_2 activity Also enhanced anti-cancer effects both in-vitro and in-vivo have been realised by combining this affinity purified anti-serum to PLA_2 components and/or mammalian PLA_2 with a non-toxic inhibitor of Phospholipase C or with anti-serum (polyclonal or monoclonal) developed to Phospholipase C enzyme.

[0024] This patent relates to the administration of one or more compounds which can generally be described as performing their fimction by either directly or indirectly causing Phospholipase A_2 and/or Phospholipase C enzyme inhibition, wherein the said inhibition is either partial or total. In addition this patent relates to the administration of one or more compounds which can generally be described as performing their function by interaction with the neoplastic cell membrane preventing their growth or spread. thus preventing further disruption of non-involved organs of the body and causing no toxicity to the infected patient or animal being treated.

[0025] Additional aspects of the invention relates to pharmaceutical compositions containing the compounds of the invention as active ingredients, modifying unwanted immune responses, and to methods of retarding proliferation of tumour cells using the compounds and compositions of this invention.

[0026] The anti-serum to snake venom PLA_2 and to plant insect, mammalian and/or to PLA_2 epitopes or mimic molecules are shown herein to be active anti-tumour proliferative compounds and immune enhancing. For use in this regard, the compounds of the invention are administered to mammals, including humans, in an effective amount of 0.05 to 5 gms per day per kilogram of body weight. The amount depends, of course, on the condition to be treated, the severity of the condition, the route of administration of the drug, and the nature of the subject. The drugs may be administered IV, orally, parenterally, or by other standard administration routes including targeting with liposomes/RBC.

[0027] The therapeutic activity of the compounds of this invention are demonstrated by inhibition of the tumour cell lines in-vitro and in-vivo. The compounds were tested for toxicity in Scid mice. Results as in **FIG. 1**[toxicity data].

[0028] Toxicity Study

[0029] Method

[0030] Female Scid mice (6-8 weeks of age) were treated with either a Neat or a 1:10 dilution of the anti-serum

preparation, subcutaneously (0.1 ml, daily) for a period of 14 days. The weights of the mice were measured daily. At termination, organs were removed and fixed in formalin for histological examination.

[0031] Results

[0032] No toxicity, as assessed by animal weights and clinical well-being, was evident (**FIG. 1**).

[0033] The compounds of this invention may be combined with other known anti-inflammatory/ immunosuppressive or chemotherapeutic agents such as steroids or non-steroidal anti-inflammatory agents in the pharmaceutical compositions and methods described herein.

[0034] Anti-serum to snake and/or insect venoms and/or mammalian and/or PLA_2 enzyme or its epitopes can be used as a therapeutic treatment in diseases where elevated levels of Phospholipase A_2 are evident, (e.g. Rheumatoid Arthritis, see Table B). It is also envisaged that this novel therapy with anti-serum to venom PLA_2 (snake or insect) and/or to PLA_2 components (derived from animal or plant) can be applied as a prophylactic therapy by using sub-lethal doses of venoms or the venom PLA_2 enzyme extracts together with mammalian or plant PLA_2 or synthetic peptides demonstrating PLA_2 activity plus adjuvant to stimulate an immunoglobulin response within the patient, see results—Vaccine Efficacy in Balb/c mice. It is also envisaged that a synthetic peptide incorporating the Phospholipase A_2 and/or Phospholipase C activity could be used to generate said anti-serum or therapeutic agent or vaccine. Use may also be made in the generating of this therapeutic vaccine/anti-serum by using the known sequence homology that exists between human Phospholipase A_2 and snake/insect venoms together with animal PLA_2 used in combination with compounds known to inhibit Phospholipase C activity or anti-serum developed to this enzyme.

[0035] Sustained or directed release compositions can be formulated, e.g. liposomes or those wherein the active compound is protected with differentially degradable coatings, e.g. by microencapsulation, multiple coatings, etc. It is also possible to freeze-dry the new compounds and use the lyophilizates obtained, for example, for the preparation of products for storage and subsequent injection.

EXPERIMENTATION

[0036] The compounds of this invention can be identified as anti-serum to snake or insect venoms mammalian or plant PLA_2 or parts thereof or Phospholipase C or mimic molecules generated to venoms or mammalian PLA_2 molecules and/or Phospholipase C or parts thereof also the pharmaceutical use of venoms or parts thereof and/or mammalian PLA_2 or enzyme components as vaccine antigen are incorporated. Non-toxic compounds showing anti-phospholipase C activity can be incorporated with the anti-serum to PLA_2 of any origin, or mimic molecules demonstrating Phospholipase A_2 activity.

[0037] In certain applications of this therapy it may be necessary to curtail the ADCC reaction which could cause serum sickness and to ensure that this does not occur the IgG (FC) component is enzymatically cleaved from the affinity purified immunoglobulin so that natural killer cells will not react to the immunoglobulin in the anti-serum.

[0038] Ability of anti-serum to snake venom to inhibit Phospholipase A_2 enzyme isolated from human synovial fluid (Table A2).

[0039] The inhibition of Phospholipase A_2 enzyme from synovial fluid isolated from a patient with Rheumatoid Arthritis was tested with a range of dilutions of anti-serum to snake venom. Anti-serum to snake venom generated in horse, reconstituted in 10 ml sterile water. The following dilutions were used 1:10, 1:20, 1:40 and 1:60. The method used was as outlined in "Infection and Immunity, September 1992, p 3928-3931. Induction of Circulating Group II Phospholipase A_2 Expression in Adults with Malaria.

TABLE A2

Results	
Dilution	Inhibition
1:10	63%
1:20	50%
1:40	35%
1:60	29%

[0040] In-vitro testing of un-affmity purified snake venom.

[0041] A range of tumour cell lines were tested with 3 concentrations of the anti-serum to snake venom by the MTT Assay. This anti-serum was not affinity purified. MTT Assay described by Alley et al, (Cancer Research, 48589-601, 1988) See Table B.

TABLE B

SUMMARY OF RESULTS	
Dilution	% of Control
Molt 4:	Human T cell Lymphoma Cancer
Serum-containing	
Neat	48.1
1:10	53.7
1:20	40.8
Serum-Free	
Neat	58.7
1:10	51.2
1:20	40.6
MDA 468:	Human Breast Cancer
Serum-containing	
Neat	8.0
1:10	53.7
1:20	58.9
Serum-Free	
Neat	15.4
1:10	48.4
1:20	58.9
C17OHM2:	Human Colon Cancer
Serum-containing	
Neat	9.3
1:10	61.4
1:20	55.6
Serum-Free	
Neat	15.2
1:10	47.3
1:20	49.5
Pan 1:	Human Pancreatic Cancer

TABLE B-continued

SUMMARY OF RESULTS

Dilution	% of Control
Serum-Containing	
Neat	9.3
1:10	47.5
1:20	49.2
Serum-Free	
Neat	43.1
1:10	53.2
1:20	69.4
841:	Human small cell lung cancer
Serum-containing	
Neat	25.2
1:10	45.5
1:20	51.1
Serum-Free	
Neat	63.4
1:10	60.1
1:20	59.8
T24:	Human Bladder Cancer
Serum-containing	
Neat	68.5
1:10	75.1
1:20	76.2
Serum-Free	
Neat	84.1
1:10	87.9
1:20	88.4

[0042] Testing un-affinity purified anti-serum to Snake Venom against B16F1 Melanoma Cell Line.

[0043] Mice

[0044] C57BL/6

[0045] Procedure

[0046] The mice were inoculated with 0.5×10^6 B16 F1 melanoma cells subcutaneously (sc) into flank region. Once palpable tumours had develcped the mice received daily sc injections as follows: -

		number of mice
A	Sterile water 100 μl	6
B	anti-serum (full strength) 100 μl	6
C	anti-serum (diluted 1:10) 100 μl	6

[0047] The dimensions of the tumours were taken daily using callipers. Once the tumours of the control mice were approximately 1.5 cm or larger in diameter all mice were killed. The tumours were removed and weighed.

[0048] Results

[0049] Small tumours were first discernible by palpitation in all mice 6-7 days after inoculation. The changes in volume as measured by callipers, together with tumour weights at autopsy. See **FIG. 2**[Effect of un-affinity purified anti-serum to snake venom on Melanoma B16F1 Growth] for effect of anti-serum to snake venom on tumour growth retardation.

[0050] IN-VITRO SREENING OF THE AFFINITY PURIFIED ANTI-SERUM TO SNAKE VENOM PREPARATION AGAINST A RANGE OF TUMOUR CELL LINES (Illustrated in **FIG. 3***A* [Human colorectal tumour C170HM2], **FIG. 3***B* [Human bladder tumour T24], **FIG. 3***C* [Human lymphoma tumour MOLT 4], **FIG. 3***D* [Human pancreatic tumour PAN 1], **FIG. 3***E* (Human breast tumour MDA 468], **FIG. 3***F* [Human small cell lung tumour 841;, **FIG. 3***G* [Human gastric ST24], and **FIG. 3***H* [Human Ovarian OVCAR3])

[0051] Introduction

[0052] The in-vitro inhibitory effects of the horse generated anti-serum to snake venom preparation, previously evaluated were obscured due to serum enhancement of tumour cell growth. Thus in the following assay, affinity purified anti-serum to snake venom was evaluated.

[0053] Method

[0054] The cell lines were seeded into 96 well plates at a cell concentration of 10^4 cells per well in both serum free (Hams F12:RPMI 1640+0.5% bovine serum albumen) and serum-containing medium (RPMI 1640+10% heat inactivated foetal calf serum). The anti-serum preparation was diluted in the corresponding medium and added to the wells, 2-3 hours after the cells (to allow for cell adherence). The plates were incubated at 37° C. in –5% $C0_2$ for 3 days. The cells were then incubated with 1 mg/ml MTT (methyl thiazol tetrazolium) for 4 hours at 37° C. The crystals were then solublised with dimethyl sulphoxide and the absorbance measured at 550 nm.

[0055] Results

[0056] The test anti-sera inhibited all of the cell lines at all concentrations examined.

[0057] The level of inhibition was statistically significant from the untreated control at all anti-serum dilutions, with all cell lines as assessed by a one way analysis of variance.

[0058] IN-VIVO TEST

[0059] The effects of affinity purified anti-serum to snake venom on human colorectal $C17OHM_2$ cell line.

[0060] Materials and Methods

[0061] $C170MH_2$ cells were injected subcutaneously into the left flank of ten male nude mice. The mice were allocated randomly to two groups.

[0062] Group 1-100μl anti-serum twice daily intravenously (IV)

[0063] Group 2-100μl PBS twice daily IV

[0064] Tumours were measured twice weekly, using callipers in two dimensions. Cross-sectional areas were calculated. The mice were also weighed once weekly. The therapy was terminated at day 22.

[0065] Results

[0066] The cross-sectional areas were measured at increasing time points during the experiment, as shown in **FIG. 4**[Effect of affinity purified anti-serum to snake venom on the mean cross-sectional area of $C170HM_2$ in nude mice].

The affinity purified anti-serum preparation induced a slowing in growth when compared to saline controls. An ANOVA was performed on the results in which the treatment was evaluated with respect to time and shows a significance of P=0.028.

[0067] At the termination of the experiment, the tumours were weighed and the results are shown in **FIG. 5**[Effect of affinity purified anti-serum to snake venom on the final tumour weight of C170HM2]. No toxic effect of the affinity purified anti-serum preparation was observed.

[0068] In-vitro screen of the affinity purified anti-serum to snake venom preparation in combination with a phospholipase C inhibitor 1-oleoyl-2- acetyl-sn-glycerol (OAG) 5μ molar, on a range of cancer cell lines.

[0069] Methods

[0070] The affinity purified anti-serum to snake venom preparation was diluted 1:2 and 1:10 and was combined with 5μ molar OAG and added to the wells as previously described for the MTT Assay. The cell lines tested were Human Breast tumour, MDA 468, Human small cell lung tumour 841 and Human renal TK-10. Results as shown in **FIG. 6***A* [Affinity purified anti-serum to snake venom and (OAG) a Phospholipase C inhibitor combination—Human breast tumour MDA 468], **FIG. 6***B* [Affinity purified anti-serum to snake venom and (OAG) a phospholipase C inhibitor combination—Human small cell lung tumour 841] and 6C [Affinity purified anti-serum to snake venom and (OAG) a phospholipase C inhibitor combination—Human renal TK-10].

[0071] In-vivo testing of the combination of affinity purified anti-serum to snake venom and 1-oleoyl-2-acetyl-sn-glyceral (OAG) at 5 μm concentration on the growth of MDA 468 cell line.

[0072] Method

[0073] MDA 468 tumours were aseptically removed from donor female Scid mice. The tissue was aseptically minced, pooled and implanted into anaesthetised female Scid mice (anaesthetic comprised of a 0.2 ml injection of Hypnorm (Jannsen) :Hyonovel (Roche) : distilled water in a 1: 1:5 ratio). Tissue implants consisted of 3-5 mm^2 pieces and after subcutaneous transplantation into the left flank, the incision was clipped. The Scid mice were then randomised into 2 groups of 10 animals. They were treated daily with a 0.2 ml subcutaneous injection (in the opposite flank to the tumour graft) of a combination of affinity purified anti-serum to snake venom and 5 μm molar of (OAG) dilution of the anti-serum preparation. The control animals received 0.2 ml phosphate buffered saline, pH 7.6. All animals were terminated on day 63, and the tumours were dissected out, weighed and processed for histology. Results are in **FIG. 7**[Effect of the affinity purified anti-serum to venom in combination with the Phospholipase C inhibitor (OAG) 5 μm].

[0074] Vaccine Efficacy in Balb/c mice after challenge with WEHI-3 cell.

[0075] The objective of study is to demonstrate the efficacy of sub-lethal levels of Russelli vipera venom entrapped in liposomes and porcine phospholipase A_2 enzyme entrapped in liposomes working in combination to confer a sustained and protective antibody response to a challenge by Leukaemia cells (WEHI-3 cells)

[0076] The Russelli vipera venom was toxoided with 2% osmium tetroxide and entrapped in liposomes (egg phosphocholine and cholesterol). The liposomes were sterilised.

[0077] The Porcine Phospholipase A_2 enzyme was entrapped in liposomes (egg phosphocholine, and cholesterol) and were sterilised.

[0078] Immunisation of mice consisted of an initial subcutaneous injection of 0.25 mls (containing 250 μg of venom) and 3 days later the mice were injected subcutaneously with 0.25 mls of porcine PLA_2 (containing 250 μg of porcine PLA_2. Boosters of each vaccine were given at 3 week intervals.

[0079] Control mice were injected with 0.25 mls of sterile physiological saline on days corresponding to test mice inoculations.

[0080] Animals

[0081] Balb/c mice (20-25 g) were used in the study. 15 mice were used in each group.

Group I	test mice
Group II	control mice

[0082] Challenge

[0083] The immunised mice and controls were challenged by intravenous injection into tail vein with approximately 5×10^5 leukemic cells (WEHI-3 cells) on day 30 of study.

[0084] Test mice are observed for extended life span after the death of the control mice after approximately 24 days.

[0085] Results Obtained

[0086] All control mice died of leukaemia within the allotted time span of 24 days. The venoid combination inoculation protected the vaccinated group from the cancer cell challenge and there was a 100% survival rate at day 35 when the experiment was terminated.

[0087] Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilise the present invention to its fullest extent. The preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the disclosure in any way whatsoever.

I claim

1. A method of treating neoplasm in a mammal in need of such treatment, comprising administering to said mammal a therapeutic agent comprising venom and/or mammalian. plant or insect anti-serum reactive with at least one Phospholipase A_2 enzyme.

2. A method according to claim 1 wherein the anti-serum is reactive with two or more Phospholipase A_2 type enzymes.

3. A method according to claim 1 wherein the at least one Phospholipase A_2 Type enzyme is Type I, Type II, Type III or Type IV.

4. A method according to claim 1 wherein the anti-serum is either polyclonal or monoclonal.

5. A method of treating a mammal prophylactically to prevent neoplastic development, comprising administering to said mammal a therapeutic vaccine containing venom and/or mammalian, plant or insect PLA_2 enzymes or part thereof as the principal antigen component.

6. A pharmaceutical formulation containing venom and/or mammalian plant or insect anti-serum to PLA_2 enzyme or part thereof in combination with anti-serum to Phospholipase C enzyme or part thereof or inhibitory compounds to

Phospholipase C for use as a therapeutic agent for the therapy of a neoplastic condition in a human or animal.

7. A method according to claim 6 wherein the inhibitory compounds to Phospholipase C is one or more of EDTA, Phenanthroline, Chloromercuribenzoic Acid, Iodoacetic Acid, and I-oleoyl-2-acetyl-sn-glycerol(OAG).

8. A pharmaceutical formulation containing one or more venoms or venom components as antigen and/or mammalian, plant or insect PLA_2 enzyme as antigen in combination with Phospholipase C enzyme.

9. A method according to claim 8 wherein the phospholipase C enzyme inhibitor is used in combination with the therapeutic agents of claim I to enhance anti neoplastic and anti metastatic activity.

10. A method according to any one of claims **1**, **5**, **6** and **8**, wherein the administration is part of a combination therapy with other therapeutically effective agents.

11. A method according to claims **1**, **5**, **6** and **8** wherein the administration is in combination with adjuvants.

12. A method according to claims **1**, **5**, **6** and **8** wherein the venom is that of snakeand/or insect.

13. A method according to claims **1**, **5**, **6** and **8** wherein the Phospholipase A_2 enzyme showing Phospholipase A_2 activity is obtained from more than one species of snake and/or insect, mammal or plant.

14. A method according to claims **1**, **5**, **6** and **8** wherein the therapeutic agent is administered as an anti-inflammatory agent.

15. A method according to claims **1**, **5**, **6** and **8** wherein the therapeutic agent is administered to prevent the occurrence of immunosuppression.

16. A method according to claims **1**, **5**, **6** and **8** wherein the therapeutic agent is administered in the treating of allergic contact dermatitis, Asthma and Psoriasis and bronchitis.

17. A method according to claims **1**, **5**, **6** and **8** wherein the anti-serum is administered for the treatment of physiological condition resultant from elevated levels of phospholipase A_2 products and/or metabolites.

18. A method according to claim 17 wherein the physiological condition is Schizophrenia.

19. A method according to claims **1**, **5**, **6**, **8** and **17** wherein the anti-serum to Phospholipase A_2 and/or C are produced synthetically by molecular imprinting of template organic molecules using these enzymes.

20. Therapeutic agents according to claims **1**, **5**, **6** and **8** for treating one or more of the following:- Rheumatoid arthritis, osteoarthritis, gout, rheumatic carditis and autoimmune diseases, allergic diseases, bronchial asthma, septic shock, renal failure, pancreatis, myasthenia gravis and ocular and dermal inflammatory diseases, psoriasis, splenomegaly, cancer, metastatic spread of neoplasm, collagen vascular disease, myocardial ischemia, cellular chemotaxis, depression, erythema, vascular permeability resultant from enhanced production of PGE_2, acne, atopic diseases, malaria, allergic conjunctivitis, schizophrenia, reiters syndrome, raynaud's phenomenon, lupus, Chron's and Graves disease.

21. A method according to claims **1**, **5**, **6**, **8** and **17** wherein the Fc receptor of the antibody to either Phospholipase A_2 and C used in this therapeutic method is either totally or partially removed.

22. A method according to claims **6**, **8**, **19** and **21** wherein a non-toxic compound demonstrating inhibiting activity against Phospholipase C enzymes may be utilised in conjunction with the PLA_2 anti-serum to enhance its anti-neoplastic (tumour) and anti-metastatic activity.

23. A method according to claims **1**, **5**, **6**, **8**,**17** and **19** wherein the anti-serum is generated to human Phospholipase A_2 enzyme either in a mono and/or polyclonal form.

24. A method according to claims **1**, **5**, **6**, **8** and **17** wherein the anti-serum to Phospholipase A_2 enzyme is generated in eggs, producing antibodies which do not react with the human Compliment system.

25. A method according to claims **1**, **5**, **6**, **8** and **17** wherein the anti-serum to venom, mammalian, plant or insect Phospholipase A_2 is generated in mammals and extracted from the colostrum and preferably but not essentially affinity purified for use in oral administration to patients either alone or in combination with anti-serum similarly produced to human Phospholipase C enzyme components.

26. A method of inoculation of human or animal with a combination of two or more phospholipase A_2 enzymes types.

27. A method according to claim 26 where the antibody response to the inoculation confers prophylactic and/or therapeutic benefit to patient.

28. A method according to claim 27 wherein the patient is in need of a treatment for a neoplastic condition.

29. A method according to claims **26**, **27** and **28** wherein the phospholipase A_2 type is Type I, Type II, Type III or Type IV.

30. A method according to claim 29 wherein the Phospholipase A_2 is obtained from venom.

31. A method according to claim 29 wherein the Phospholipase A_2 is obtained from animal or plant species.

32. A method according to claim 1, **5**, **6**, **8** and **26** wherein the phospholipase A_2 is synthetically produced or cloned.

33. A method of early detection of neoplastic disease by utilising the detection of enhanced PLA_2 levels in patients.

34. A method according to claim 33 wherein the detection of enhanced PLA_2 is established by the use of Lipose Diagnostic Kit.

35. A method according to claims **2**, **26**, **27** and **28** wherein Phospholipase A_2 type enzyme has a size of between 40-80 kDa.

36. A method of targeting cancer cells by use of Type I and/or Type II PLA_2 as targeting agent with hydrophilic tail.

37. A method according to claim 36 wherein the targeting agent is a liposome containing anti-serum to PLA_2 or conventional chemotherapy drugs.

38. A method treating parasitic and bacterial infections in mammals by the administration of a therapeutic agent containing venom and/or mammalian, plant or insect anti-serum reactive with Phospholipase A_2 enzymes

39. A method according to claim 38 wherein the anti-serum is reactive with one or more Phospholipase A_2 type enzymes

40. A method according to claim 39 wherein the Phospholipase A_2 Type enzymes is one of Type I, Type II, Type III or Type IV.

41. A method according to claim 38 wherein said parasite is an haemoflagellate parasite.

42. A method as recited in claim 41 wherein said parasite is a member of the group of haemoflagellate parasites consisting of Leishmania, Trypanosomia and Toxoplasma.

* * * * *