Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2382293 A1 2001/02/22

(21) 2 382 293

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2000/08/18
(87) Date publication PCT/PCT Publication Date: 2001/02/22
(85) Entrée phase nationale/National Entry: 2002/02/18
(86) N° demande PCT/PCT Application No.: US 2000/022610
(87) N° publication PCT/PCT Publication No.: 2001/012205
(30) Priorité/Priority: 1999/08/18 (60/149,429) US

(51) Cl.Int.[7]/Int.Cl.[7] A61K 33/40, A61P 35/00
(71) Demandeur/Applicant:
OXO CHEMIE AG, CH

(72) Inventeurs/Inventors:
KUHNE, FREDERICH W., TH;
KODELJA, VITAM, US

(74) Agent: BERESKIN & PARR

(54) Titre : SOLUTIONS DE CHLORITE CHIMIQUEMENT STABLES, DESTINEES AU TRAITEMENT DU CANCER ET D'AUTRES MALADIES

(54) Title: CHEMICALLY-STABILIZED CHLORITE SOLUTIONS FOR TREATING CANCER

(57) Abrégé/Abstract:
Methods of using a stabilized chlorite matrix to modulate immune responses and treat cancer are disclosed. The stabilized chlorite matrix, when administered to a mammal in need thereof, can activate immune cells in a manner similar to interferon gamma but does not effect the production of inflammatory and shock related cytokines like tumor necrosis alpha. The stabilized chlorite matrix also upregulates the expression of the DCC protein in macrophages, a protein whose expression is related to neoplastic transformation. By activating macrophages, the stabilized chlorite matrix therefore is useful as an immunomodulatory agent and in treating cancer.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 February 2001 (22.02.2001)

PCT

(10) International Publication Number
WO 01/12205 A2

(51) International Patent Classification[7]: A61K 33/40, A61P 35/00

(21) International Application Number: PCT/US00/22610

(22) International Filing Date: 18 August 2000 (18.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/149,429 18 August 1999 (18.08.1999) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 60/149,429 (CIP)
Filed on 18 August 1999 (18.08.1999)

(71) Applicant *(for all designated States except US)*: **OXO CHEMIE AG** [CH/CH]; 4, rue Georges-Jordil, CH-1701 Fribourg (CH).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KUHNE, Frederich, W.** [DE/TH]; 155/1 Mooban Panya Soi 23, Pattanakarn Road, Bangkok 10250 (TH). **MCGRATH, Michael** [US/US]; Oxo Chemie, Inc., Suite 450, 601 Gateway Boulevard, South San Francisco, CA 94080 (US). **ENGLEMAN, Edgar, G.** [US/US]; Oxo Chemie, Inc., Suite 450, 601 Gateway Boulevard, South San Francisco, CA 94080 (US).

(74) Agents: **SANDERCOCK, Colin, G.** et al.; Foley & Lardner, 3000 K. Street, NW, Washington, DC 20007-5109 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CU, CZ, DE, DK, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/12205 A2

(54) Title: CHEMICALLY-STABILIZED CHLORITE SOLUTIONS FOR TREATING CANCER AND OTHER DISEASES

(57) Abstract: Methods of using a stabilized chlorite matrix to modulate immune responses and treat cancer are disclosed. The stabilized chlorite matrix, when administered to a mammal in need thereof, can activate immune cells in a manner similar to interferon gamma but does not effect the production of inflammatory and shock related cytokines like tumor necrosis alpha. The stabilized chlorite matrix also upregulates the expression of the DCC protein in macrophages, a protein whose expression is related to neoplastic transformation. By activating macrophages, the stabilized chlorite matrix therefore is useful as an immunomodulatory agent and in treating cancer.

5 # CHEMICALLY-STABILIZED CHLORITE SOLUTIONS FOR TREATING CANCER AND OTHER DISEASES

The present invention relates to stabilized chlorite matrices as immunomodulatory agents and anti-cancer agents. The stabilized chlorite
10 matrix inhibits can affect the activation of macrophages in a manner similar to interferon gamma but without affecting the release of cytokines that cause side effects, such as tumor necrosis alpha. The stabilized chlorite matrix also upregulates the expression of the DCC protein in macrophages, a protein whose expression is related to neoplastic transformation. By
15 activating macrophages, the stabilized chlorite matrix therefore is useful as an immunomodulatory agent and in treating cancer.

A feature common to an immune response is the recognition of an antigen (either foreign or self, but perceived as foreign), and subsequent processing by the immune system. Typically, antigen is degraded by
20 enzymes in the cytoplasm, endoplasmic reticulum (ER) and lysosomes of cells, (usually macrophages, dendritic cells and other antigen presenting cells (APCs)), or in serum. The degraded antigen is presented on the surface of the APC by MHC class I or II molecules. This presentation of the antigenic epitope by the MHC molecule, and subsequent binding to the T
25 cell receptor (TCR) of a T cell is known as antigen presentation. Rodgers, J.R., *et al.*, CLINICAL IMMUNOLOGY, PRINCIPLES AND PRACTICE (RICH): "Antigens and antigen presentation," Chpt. 7, pp 114-131, Mosby, St. Louis, MO (1996).

Modulation of the immune response has been achieved by treatment
30 with any of a variety of cytokines or lymphokines. In particular, interferons (IFNs) are type of cytokine that plays a complex and central role in the resistance of mammalian hosts to pathogens and has been found useful in cancer therapy. Type I interferon (IFN-α and IFN-β) is secreted by virus

infected cells, while type II interferon (IFN-χ) is secreted by thymus-derived cells under conditions of activation and by natural killer cells (NK cells).

Among others, IFN-χ has an ability to inhibit cell proliferation at much lower concentrations comparing with IFN-α, and IFN-β. (Rubin, B. Y. et al.
5 (1980): Proc. Natl. Acad. Sci. U.S.A., 77, 5928), and also to activate cells, including natural killer cell, killer T-cell, K-cell and macrophage, which have cancer therapeutic effects J. L. Crane et al., J. Natl. Cancer Inst., 61, 871-874, 1978; J. E. Blalock et al., Cell Immunol., 49, 390-394, 1980 (reporting that the antitumor activity of gamma-interferon is 20 to 100 times as high as
10 those of alpha- and beta-interferons). U.S. Patent No. 5,268,169 describes the use of IFN-χ for treating ovarian carcinoma. The use of purified recombinant interferon in cancer therapy and in modulating the immune response has been of limited success because of the high costs of producing recombinant material and because IFN-χ causes side effects
15 which occur from the stimulation of inflammatory and shock related cytokines such as TNF-α. Furthermore, administration of protein compounds such as cytokines and elicit a detrimental immune response to the administered protein.

Aqueous solutions of a chemically stabilized chlorite matrix that are
20 capable of intravenous administration are known. Other chlorine-containing solutions also are known to have reported medicinal uses. For example, United States Patent No. 5,019,402 discloses a solution containing chlorine dioxide or a chlorine dioxide-liberating mixture of a chlorite, a weakly acidic buffer and a heat-activated saccharide which can be used for the
25 sterilization of stored blood components with the exception of those which contain red blood corpuscles, *i.e.*, of leukocytes, blood platelets, coagulation factors and globulins. In whole blood, a corresponding disinfecting action does not occur, presumably because the red blood corpuscles are attacked more quickly by the chlorine dioxide than the

targeted micro-organisms. Therefore, this agent also is not suitable for a parenteral administration.

DE-OS 32 13 389, United States Patent No. 4,507,285 and United States Patent No. 4,296,103, describe chemically-stabilized chlorite matrices which are suitable for an external or oral therapeutic use. Besides various bacterial infections, the external treatment of virus infections, such as herpes simplex and herpes zoster, may be possible in this manner, but the documents do not report the use of these chlorite matrices for intravenous administration for inhibiting an antigen-specific immune response.

European Patent EP 0 200 157 and United States Patent No. 4,725,437 further describe solutions of a chemically-stabilized chlorite matrix for intravenous and perioperative administration. The agent has proved to be effective in the treatment of *Candida albicans* infections. From EP 0 200 157, it is known to use such stabilized chlorite matrices for intravenous and/or local administration in cases of infectious conditions brought about by parasites, fungi, bacteria, viruses and/or mycoplasts. The action is explained by a phagocyte stimulation which is achieved by a single effective administration of the chlorite complex shortly after the infection. PCT publication WO 99/17787, which is hereby incorporated by reference in its entirety, discloses chemically-stabilized chrlorite solutions for inhibiting antigen-specific immune responses.

Providing an approach to cancer therapy that is similar to that achieved by interferon gamma but yet avoids the production of toxic substances such as TNF alpha is needed. Thus, there exists a need to develop a method of treating cancer and other malignancies using a substance that has the macrophage activating properties of IFN-χ while lacking the inflammatory effects and toxic shock effects of this molecule such as occur by the activation of the expression of TNF-α. It is therefore an object of the invention to provide a method of treating cancer by

administration of an inorganic compound that has immunomodulatory properties. It is also an object of the invention to provide a method of treating cancer by activating macrophages from an individual with cancer ex vivo with a non-protein IFN-χ and then administering the activated cells into
5 the individual.

In accordance with these and other objects of the invention, there is provided a method of treating cancer and other malignancies in a animal, such as a mammal, comprising administering an effective amount of an aqueous solution comprising a stabilized chlorite matrix. The method
10 activates *inter alia* macrophages resulting in upregulation of the expression deleted in colon carcinoma (DCC) protein. An effective amount is 5 to 100 mMol of ClO_2^- per liter of solution.

In one embodiment of the present invention, the cancers for which the method is used are those cancers characterized by a reduced
15 expression of the DCC protein.

In another embodiment of the present invention, the cancer cells to be treated are selected from the group consisting of colon carcinoma, gastric carcinoma, esophageal carcinoma, rectal carcinoma, pancreatic carcinoma, prostate carcinoma, glioma and neuroblastoma.

20 In accordance with an additional object of the present invention, there is provided methods of treating cancer or other malignancies in an animal, such as a mammal, wherein the methods comprise (a) removing macrophages from the mammal; (b) contacting the macrophages with an effective amount of an aqueous solution comprising a stabilized chlorite
25 matrix under conditions sufficient to increase the expression of DCC on the macrophages; and (c) introducing the macrophages from step (b) back into the mammal. An effective amount is 5 to 100 mMol of ClO_2^- per liter of solution.

In one embodiment of the present invention, the cancers for which the method is used are those cancers characterized by a reduced expression of the DCC protein.

In another embodiment of the present invention, the cancer cells to 5 be treated are selected from the group consisting of colon carcinoma, gastric carcinoma, esophageal carcinoma, rectal carcinoma, pancreatic carcinoma, prostate carcinoma, glioma and neuroblastoma.

Further objects, features and advantages of the present invention will become apparent from the detailed description of preferred embodiments 10 that follow.

The chlorite matrix solutions of the present invention can be dosed *in vivo* corresponding to the body weight, whereby, because of the continuous breakdown of the active material in the blood, the agent must be 15 administered again at regular intervals. Those skilled in the art are capable of varying the concentrations of antigen-presentation-inhibiting solutions depending on available *in vitro* data and body weight. Thus, throughout the specification and claims, the phrase "an effective amount" will be known by those skilled in the art to mean an amount of solution which, when 20 administered *in vivo* to subjects of varying weight, will bring about modulation of the immune response, and consequently, activation of macrophages and other immune cells. Typically, an inhibition effective amount of the chlorite matrix solution will vary between about 0.1 ml/kg to about 1.5 ml/kg, preferably, about 0.5 ml/kg of body weight and at a 25 concentration of about 40 to about 80 mMol ClO_2^- per liter, preferably about 60 mMol ClO_2^- per liter, respectively.

Preferably, the chlorite matrix solution of the invention is administered once daily for anywhere from about three to seven days, preferably five days, followed by a period of rest of from 10 to 20 days, 30 preferably from 14-18 days, and more preferably, 16 days, to constitute one

cycle of treatment. Preferably, patients are treated with more than one cycle, more preferably, at least three cycles, and most preferably, at least five cycles.

An alternative treatment regimen consists of intravenously administering the chlorite matrix solution of the invention once daily for a period of five days, followed by two days of rest (*i.e.*, over the weekend), followed by five more consecutive days of administration, followed by a period of rest from anywhere between 1 and 4 weeks to constitute one cycle. Preferably, patients are treated with more than one cycle, more preferably more than three. Skilled artisans are capable of modifying the administration of the stabilized chlorite matrix of the invention depending on the disease treated and the size of the patient, using the guidelines provided herein.

The use of an aqueous solution containing a stabilized chlorite matrix for treating wounds and infections was known in the art. United States Patent Nos. 4,507,285 and 4,725,437 to Kühne, the disclosures of which is incorporated by reference herein in their entirety, and EP 0 200 157 to Kühne, the disclosure of which also is incorporated by reference herein in its entirety, describe the use of a stabilized chlorite matrix solution in stimulating the wound healing response in humans, as well as treating infections caused by parasites, fungi, bacteria, viruses and/or mycoplasma. Kühne *et al.*, European Patent No. 200,156, the disclosure of which also is incorporated by reference herein in its entirety, describes the use of a stabilized chlorite matrix solution in conjunction with radiation therapy to aid in repairing damaged irradiated tissue and reducing side effects. The mode of action in treating damaged and/or infected tissue is described as amplifying the "oxidative burst" response of phagocytes in the presence of bioactivators, e.g., heme compounds. Wound healing and treating the reported infections can be effected in general by activating macrophages present in the body which in turn serve to activate fibroblast cells which

stimulate the wound healing response. The stabilized chlorite matrix solutions can activate macrophages by complexing with the heme moieties present in the macrophage membrane. Upon activation, the macrophages stimulate the fibroblast cells which in turn generate collagen and endothelial cells that are useful in repairing damaged tissue caused by the wound or by the infections. While not intending on being bound by any theory, the present inventors believe that a macrophage is stimulated by the stabilized chlorite matrix solution by the following sequence of events. The formula for the stabilized chlorite matrix can be summarized as $(ClO_2^-)_n \times O_2$, where n is between 0.1-0.25, preferably, about 0.21. In the presence of heme compounds (*e.g.*, hemoglobin, myoglobin, peroxidases, cytochromes, etc.), which are present in the serum as biological molecules, and they are part of the cell membrane of phagocytic cells like macrophages, the stabilized chlorite matrix becomes a secondary oxidant with oxidative properties different from chlorite and hydrogen peroxide. Indeed, the stabilized chlorite matrix of the invention has shown pharmacological differences when compared to equimolar chlorite solutions. (Ivankovic *et al.*, 1993).

Macrophage activation brought about by the chlorite matrix occurs via a mechanism that is different from that of other macrophage-activating agents such as PMA, TNF-α, etc. Macrophages can be activated by a stabilized chlorite matrix because the matrix acts as an oxidant in a drug-membrane interaction which is believed to cause an electron deficiency (as a result of electron-reduction $^{+3}ClO_2^- + 4e^- \rightarrow {}^{-1}Cl^-$) on the surface of the cell. The membrane of the macrophage now has a charged surface. The present inventors believe that this charged surface may have an influence on surrounding particles (*e.g.*, invading bacteria) and/or may trigger a signal sequence into the cell. The activated macrophage then increases the DNA-synthesis of fibroblasts which is believed to contribute to its wound healing and/or parasite, bacteria, etc. infection healing properties.

The present inventors believe further that the known wound-healing mechanism via macrophage activation of the chlorite matrix of the invention also stimulates and enhances the phagocytic activity of the macrophage. Thus, the activated macrophage is primed to ingest, digest and dispose of
5 foreign antigens. The use of the claimed stabilized chlorite matrix to render macrophage phagocytic also was known and described in EP 0 200 157 to Kühne.

What was not known, however, was that a stabilized chlorite matrix also can inhibit an antigen-specific immune response, while at the same
10 time enhance the activity of phagocytes. While not intending to be bound by any theory, the present inventors believe that the stabilized chlorite matrix, when administered to a mammal in need thereof, partially or completely impedes the antigen presentation of antigen presenting cells (APCs). Throughout this description, the expression, "antigen presenting
15 cells" denotes a cell that is capable of presenting an antigen and eliciting an immune response. Useful antigen presenting cells include macrophages and dendritic cells. That administration of WF-10 prevents and/or impedes antigen presentation has been confirmed by *in vitro* data described in the examples, and is consistent with *in vivo* data also described in the
20 examples.

A typical immune response involves stimulating a macrophage, the stimulated macrophages present MHC Class I and II antigens on surface, which, when coupled with the T cell receptor, will stimulate T cells (typically a T cell subset such as CD4 or CD8 cells, and the like) to proliferate and
25 form cytotoxic T-lymphocytes (CTL) cells which in turn kill cells expressing the antigen. After antigen presentation and upon coupling with the T cell receptors, the stimulated APC (macrophage and the like) also secretes various cytokines that can aid in the proliferation of CTLs. Cytokines, or growth factors, are hormone-like peptides produced by diverse cells and are
30 capable of modulating the proliferation, maturation and functional activation

of particular cell types. Herein, cytokines refer to a diverse array of growth factors, such as hematopoietic cell growth factors (e.g., erythropoietin, colony stimulating factors and interleukins), nervous system growth factors (e.g., glial growth factor and nerve growth factor), mostly mesenchymal growth factors (e.g., epidermal growth factor), platelet-derived growth factor, and fibroblast growth factor I, II and III, including interferons.

It will be appreciated that there may be several cytokines that are involved in inducing cell differentiation and maturation, and that cytokines may have other biological functions. In the case of IL-1, there may be several forms, such as IL-1-alpha and IL-1-beta, which nevertheless appear to have a similar spectrum of biological activity. Those cytokines that are primarily associated with induction of cell differentiation and maturation of myeloid and possibly other hematopoietic cells include, *inter alia*, IL-1, G-CSF, M-CSF, GM-CSF, Multi-CSF (IL-3), and IL-2(T-cell growth factor, TCGF). IL-1 appears to have its effect mostly on myeloid cells, IL-2 affects mostly T-cells, IL-3 affects multiple precursor , G-CSF affects mostly granulocytes and myeloid cells, M-CSF affects mostly macrophage cells, GM-CSF affects both granulocytes and macrophage. Other growth factors affect immature platelet (thrombocyte) cells, erythroid cells, and the like.

When an antigen is presented to a patient with a normal, or uncompromised, immune system, the following sequence of events typically takes place. The antigen (or foreign body) is enclosed in vesicles in the macrophage which serves as a type of garbage disposal to break or digest the foreign matter down into smaller peptides. An MHC class II molecule will transport one of the smaller antigenic peptides to the surface of the macrophage and present it to a T cell receptor (TCR). Binding with the cell receptor will trigger the release of activating factors and cytokines such as IL-1, TNF, etc., which restores the self-defense of the macrophage and enhances the intracellular killing of the foreign body. If binding does not occur, the activating factors will not be released and the macrophage will

not know to digest or break the foreign matter down into smaller peptides. As it is used in this description, the expression "antigen presentation" therefore denotes the process of presentation of an MHC Class II the surface of an APC followed by subsequent binding with a TCR.

5 The present inventors believe that binding of the T-cell to the presented antigen is effected not only by recognition of the antigenic peptide that is presented, but also by the presence of the CD28 antigen on the surface of the T cell and its binding with B7. Binding of B7 to CD28 is believed to costimulate T cell proliferation, cytokine stimulation, cytokine 10 production and proliferation of cytotoxic T cells (CTL). Rudd, C.E., "Upstream-Downstream: CD28 Cosignaling Pathways and T Cell Function," *Immunity*, : 527-34 (1996); Fagnoni, F.F., *et al.*, "Role of B70/B7-2 in $CD4^+$ T-Cell Immune Response Induced by Dendritic Cells," *Immunology*, : 467-74 (1995). While not intending on being bound by any theory, the inventors 15 believe that one of the mechanisms by which antigen presentation can be prevented is by preventing binding of B7 and CD28. This can take place by either preventing presentation of B7, or by increasing the quantity of a specific subset of T cells $^-$, such as ($CD3^+$, $CD8^+$, $CD28^-$). While there is evidence showing an increase in $CD28^-$ T-cell subset for $CD8^+$ cells *in vivo*, 20 it is believed that administration of the stabilized chlorite matrix of the invention also will produce an increase in the $CD28^-$ T-cell subset for $CD4^+$ T-cells. Thus, the inventors believe that one of the mechanisms by which administration of a stabilized chlorite matrix operates to prevent and/or inhibit antigen presentation is by preventing binding of the antigen 25 presented by the APC to the TCR, and hence, preventing secretion of cytokines and other activating factors, and preventing proliferation of T cells.

In contrast to the normal immune response, if no B7 is present, or the T cell is a subset including $CD28^-$, then the present inventors believe that an anergic response will ensue. In this context, when the APC 30 presents the MHC Class II antigen, the T cell will not proliferate, nor will the

APC release cytokines and other activating factors, even though the TCR on the T cell recognizes the antigen. While not intending on being bound by any theory, the present inventors believe that administration of an aqueous solution of a stabilized chlorite matrix results in an increase in concentration of $CD28^-$ T cell subsets and/or a decrease in B7 presentation in APCs. As a consequence, there is no B7-CD28 interaction between the APC and the TCR, and therefore no T cell proliferation or cytokine release.

Previously known therapies for preventing T cell proliferation typically acted on cytotoxic T-cells after cytokine stimulation. For example, cyclosporin A is believed to act on the cytotoxic T-Lymphocyte to prevent T-cell proliferation. At this point, however, the APC already has released cytokines that might assist CTL proliferation. Accordingly, a significant amount of these drugs must be administered to prevent the CTL proliferation. There are no known methods for impeding an immune response, however, where the APC or TCR are affected in a manner that partially or completely interrupts the antigen presentation interaction between the APC and the T cell.

Patients suffering from autoimmune diseases and diseases caused by inappropriate immune response such as myasthenia gravis, systemic lupus erythematosis, serum disease, type I diabetes, rheumatoid arthritis, juvenile rheumatoid arthritis, rheumatic fever, Sjörgen syndrome, systemic sclerosis, spondylarthropathies, Lyme disease, sarcoidosis, autoimmune hemolysis, autoimmune hepatitis, autoimmune neutropenia, autoimmune polyglandular disease, autoimmune thyroid disease, multiple sclerosis, inflammatory bowel disease, colitis, Crohn's disease, chronic fatigue syndrome, and the like, do so because the immune response is inappropriate. That is, the patient's body is producing too many CTLs, or other cytokines which turn against the body's own healthy cells and destroy them. In transplant or graft patients, an inappropriate immune response occurs because the immune system recognizes the transplanted organ or

graft's antigens as foreign, and hence, destroys them. Likewise, transplant and graft patients can develop a graft vs. host response where the transplanted organ or graft's immune system recognizes the host's antigen as foreign and destroys them. This results in other inappropriate immune responses include excess inflammation, allergic asthma, allergic rhinitis and atopic dermatitis.

Conventional therapies for autoimmune disease and transplant rejection invoke application of cytotoxic agents, particularly those that affect the lymphoid system (and therein particularly the T-lymphocytes), to depress host immunity in certain autoimmune diseases, e.g., systemic lupus erythematosis, and in patients receiving organ transplants. These cytotoxic drugs are similar to those often used in cancer chemotherapy, with the attendant myeloid and other hematopoietic side effects. In addition to these drugs, specific antibodies against these lymphoid cells (particularly T-cells), e.g., the anti-Tac monoclonal antibody of Uchiyama et al., J. Immunol. 126:1393 and 1398 (1981), which specifically binds to the human IL-2 receptor of activated T-cells, can be conjugated to cytotoxic agents, such as drugs, toxins or radioisotopes, to effect a relatively select killing of these cells involved in organ rejection. For example, a T-cell antibody can be conjugated with a beta- or alpha-emitting radioisotope, and this can be administered to the patient prior to undertaking organ transplantation and, if needed, also thereafter. In order to effect a high T-cell killing dose without the concomitant limiting side effects to the hematopoietic system, the treatment using the aqueous solution containing a stabilized chlorite matrix can be used instead of, or in conjunction with any of the aforementioned agents.

Administering an aqueous solution containing a stabilized chlorite matrix to a mammal can therefore inhibit antigen-specific immune responses, while at the same time not compromise the immune system entirely because the solution also is effective in enhancing phagocytic

activity. Thus, the invention encompasses methods of treating auto-immune diseases, preventing transplant organ or graft rejection and septic shock as a result thereof, and reducing inappropriate immune responses such as excessive inflammation and allergic reaction. Because there are other methods already known to treat these disorders, skilled artisans are capable of modifying the known techniques by administering an inhibition effective amount of an aqueous solution containing a stabilized chlorite matrix, using the guidelines provided herein. For example, skilled artisans are capable of designing a treatment regimen to treat any of the aforementioned disorders using the stabilized chlorite matrix of the invention by varying the dosage amount, frequency of administration, or mode of administration.

A preferred embodiment of the treatment of this invention entails administration to a mammal in need thereof, an aqueous solution of a product that has become known as "tetrachlorodecaoxygen anion complex," commonly abbreviated as "TCDO," which is a product of Example 1 of U.S. Patent No. 4,507,285. The product is a water clear liquid, miscible with alcohols, having a melting point of -3°C. The Raman spectrum shows bands of 403, 802 (chlorite) and 1562 cm^{-1} (activated oxygen).

The DCC (deleted in colon carcinoma) gene, which contains more than one million base pairs, is a cell surface molecule receptor that is considered to function in tumor suppression. Reduction in the expression of the DCC gene has been observed in Stage 2 and stage 3 carcinomas, indicating relationship between loss of expression and survival. DCC is related to the neural cell adhesion molecule. Reduced expression in the level of DCC has been implicated in poor prognosis in gastrointestinal cancers by Saito et al., Oncology 56(2):134-41 (1999).

The present invention provides a method of treating cancer in a mammal comprising administering an aqueous solution comprising a stabilized chlorite matrix. This method involves activation of macrophages

in a manner similar to that of IFN-χ, but with release of lower amounts of inflammatory and shock cytokines such as TNF-α. The administration of the stabilized chlorite matrix results in increased expression of the DCC protein on macrophages.

5 The method of treating cancer with stabilized chlorite matrix is useful in cancers that are characterized by a reduced expression of the DCC protein. Such cancers include, for example, colon carcinoma, gastric carcinoma, esophageal carcinoma, rectal carcinoma, pancreatic carcinoma, prostate carcinoma, glioma and neuroblastoma.

10 The present invention also provides a method of treating cancer in a mammal comprising: (a) removing macrophages from the mammal; (b) contacting the macrophages with an effective amount of an aqueous solution comprising a stabilized chlorite matrix under conditions sufficient to increase the expression of DCC on the macrophages; and (c) introducing 15 the macrophages from step (b) back into the mammal. In this method the treated macrophages exhibit and activated by the stabilized chlorite matrix and increase the expression of the DCC antigen on the macrophages. Administration of such activated macrophages is useful for treating cancers characterized by a reduced expression of the DCC protein.

20 The invention now will be explained in detail with reference to the following examples. In the examples, "WF10" denotes an aqueous solution of TCDO.

Example 1

25 In this example, and the following examples 2-4, details regarding the methods used in performing these examples can be found in Fagnoni, F.F., *et al.*, "Role of B70/B7-CD28 in CD4^{+} T-Cell Immune Response Induced by Dendritic Cells," *Immunology*, : 467-74 (1995), the disclosure of which is incorporated herein by reference in its entirety. This example, 30 together with the following examples 2-4, elucidate the role of WF10 in

inducing anergy by preventing dendritic cell-mediated costimulation at the B7/B70-CD28 interface.

Dendritic cells, T cells and monocytes were obtained in the manner described in Fagnoni *et al.* To assess the effects of WF10 on DC-
5 dependent T cell activation, freshly isolated $CD4^+$-T cells were activated with allogeneic MLR in the presence or absence of WF10 to DC. Purified resting $CD4^+$ T cells (5-10 $\times 10^4$/well) were cultured with irradiated (25 Gy) allogeneic DC in U-bottomed 96-well plates containing 200 μl of complete medium. The cultures were carried out at 37°, 8% CO_2 in humidified air for
10 5 days. Cultures were pulsed with 1 μCi [^{3}H]thymidine (6-7 Ci/mm, New England Nuclear, Boston MA) 19 hour before harvest. The [^{3}H]thymidine incorporation by proliferating cells was measured in a ß-scintillation counter. WF10 was added to DC stimulated allo-MLR DC and incubated at 4° for about 3 minutes before the addition of $CD4^+$ T cells. The $CD4^+$ T cell
15 response to DC stimulated allogenic MLR was inhibited in a dose-dependent manner by WF10. The WF10 was administered by adding WF10 to culture medium at time 0 in doses of 25 μg/ml or 50 μg/ml. As the number of dendritic cells (DC) per well was increased, the number of CPM + SE (counts per minute + standard error) remained essentially the same,
20 with the greatest degree of inhibition resulting from WF10/1600. The expression WF10/number denotes that dilution of WF10 and designates the amount of WF10 per ml of solution. For example, WF10/1600 denotes a diluted solution of WF10 containing 1 ml of WF10 per 1600 ml of solution.

Example 2

Example 1 was repeated with the exception that Adherent monocytes, obtained in accordance Fagnoni *et al.* were used instead of DC. 5 Administration of WF10 was effective in inhibiting proliferation of $CD4^+$ T-cells from monocyte stimulated MLR. Indeed, with administration of WF1/1600, the stabilized chlorite matrix was effective in completely inhibiting proliferation of $CD4^+$ T-cells from monocytes stimulated with allogeneic MLR, despite increased concentration of monocytes per well.

10 The results of examples 1 and 2 therefore show that WF10 is effective in inhibiting proliferation of $CD4^+$ T cells from DC or monocytes stimulated with allogeneic MLR.

Example 3

15

Examples 3 and 4 were carried out to determine the effect of WF10 on the inhibition of antigen-induced proliferation of T cells using various antigens. In this example, purified resting $CD4^+$ T cells (5-10 $\times 10^4$/well) were cultured with irradiated (25 Gy) autologous DC in U-bottomed 96-well 20 plates containing 200 μl of complete medium. The cultures were carried out at 37°, 8% CO_2 in humidified air for 6 days. Cultures were pulsed with 1 μCi [^{3}H]thymidine (6-7 Ci/mm, New England Nuclear, Boston MA) 19 hour before harvest. The [^{3}H]thymidine incorporation by proliferating cells was measured in a ß-scintillation counter.

25 Soluble keyhole limpet hemocyanin (KLH) and tetanus toxoid (TT) were added to autologous DC. Measurements were taken for no addition of WF10, addition of WF10/200 and WF10/800 (representing administration of WF10 to the culture medium at time 0 of 0, 1ml/200 ml of solution and 1 ml/800 ml of solution, respectively) to determine the proliferation of $CD4^+$ T 30 cells when no antigen, TT, KLH25 (25 μg/ml) and KLH50 (50 μg/ml) were

presented by DC. The number of proliferated T-cells for samples using no WF10, and for samples using WF10 show significant proliferation of $CD4^{+}$ T cells occurred when DC presented the soluble antigens KLH and TT. Administration of WF10, however, almost completely inhibited the
5 proliferation of $CD4^{+}$ T cells when either KLH or TT were presented by DC.

Example 4

Example 3 was repeated except that monocytes were used instead
10 of DC for antigen presentation. In addition, WF10 was administered in the following increments WF10/200, WF10/400, WF10/800 and WF10/1600. The results show tha there was significant proliferation of $CD4^{+}$ T cells when monocytes presented the soluble antigens KLH and TT. Administration of WF10, however, almost completely inhibited the proliferation of $CD4^{+}$ T cells
15 when either KLH or TT were presented by monocytes.

The results achieved by administration of an aqueous solution containing a stabilized chlorite matrix reveal that it is capable of inhibiting an antigen-specific immune response. It has previously been reported that administration of an aqueous solution containing a stabilized chlorite matrix
20 is effective in enhancing phagocytic activity. Thus, it now is possible by administering only one medicament to inhibit one immune response, (antigen presentation and proliferation of T cells) while at the same time, enhance another immune response (phagocytosis).

25 Example 5

A phase 2 trial was conducted at San Francisco General Hospital. The study enrolled 18 patients in an open label pathogenesis study of WF-10. Patients received one hour infusions of WF-10 for one week, followed
30 by two weeks of rest. On the third week, the patients again received one

hour infusions of WF-10 daily for one week followed by two weeks of rest. Parameters studied included measures of macrophage activation/function immunologic activation and HIV viral load. RBC hemolysis evaluation studies included 51 Cr-RBC survival studies compared with changes in 5 hemoglobin, haptoglobin and reticulocyte values.

There were no side effects noted in any of the 18 patients. Data on eight of the patients were gathered and the results are tabulated below. There appeared to be acute increases in the following parameters as measured by flow cytometry (FACSCAN as recommended by, for example, 10 Becton-Dickinson) in relation to drug administration, changes that generally returned close to baseline within 2 weeks of drug administration: CD-4, CD-8, $CD14^+/CD69^+$, $CD14^+$ side scatter, $CD20/DR^+$ cells. Several values seemed to generally increase through the study, showing no clear downward trend by the end of the study and may represent long-term 15 changes induced by WF-10. These include an increase in macrophage phagocytosis index and an increase in the $CD3^+/CD8^+/CD28^-$ subset of T-cells.

Potential downward trends were noted in the following categories: macrophage intracellular TNF-α secretion, and a decrease in the number of 20 circulation $CD14^+/DR^+$ cells. It has been reported that immune paralysis results when the number of circulating $CD14^+/DR^+$ cells decreases to such an extent to reach a threshold value. No obvious changes were noted in T-cell PHA activation values or HIV load as measured by the HIV bDNA assay (most of the patients had no detectable HIV thoughout the study). Results 25 of the RBC survival studies showed no evidence for hemolysis in response to the treatment.

Administration of WF10 results in an increase in $CD14^+/CD69^+$ cells, with dramatic increases immediately following infusion. A decrease in $CD14^+/TNF$ secretion after administration of WF10 was seen thereby

indicating that the stabilized chlorite matrix of the invention is effective in decreasing secretion of the tumor necrosis factor cytokine.

Administration of WF10 to patients *in vivo* results in a steady increase in the number of $CD3^+/CD8^+$, as well as a steady increase in the
5 number of $CD3^+/CD8^+/CD28^-$ T cells. The *in vitro* data above shows inhibition of antigen presentation using $CD4^+$ T cells, and an increase in the number of circulating $CD28^-$ T cells ($CD3^+$ T cells). Thus, the inventors believe that one of the possible mechanisms by which the stabilized chlorite matrix of the invention inhibits and/or prevents antigen presentation may be
10 by blockage of the B7/CD28 interaction between the APC and the TCR.

An increase in phagocytosis index is observed upon administration of WF10. A decrease in immune function upon administration of WF10 by virtue of the decrease in $CD14^+/DR^+$ cells also is observed. The inventors therefore believe that the stabilized chlorite matrix of the invention is
15 capable of up-regulating phagocytosis, while at the same time, down-regulating or suppressing the cell-mediated and humoral immune response.

The results tabulated below summarize the data from 15 patients and show the changes in the measured parameter between the 8th day and the 47th day of treatment. The 8th day represents the first day of WF10
20 administration because the first 7 days of treatment are devoted to patient evaluation.

CD3$^+$, CD8$^+$, CD28$^-$	0.027	increase
CD14$^+$, TNF-	0.017	decrease
CD14$^+$, DR$^+$	0.032	decrease
CD3$^+$, CD4$^+$, CD38$^+$ (MF CD38 Antigen)	0.021	decrease
CD3$^+$, CD8$^+$, CD28$^+$ (MF CD28 Antigen)	0.010	decrease
CD20$^+$, DR$^+$ (MF DR Antigen)	0.014	decrease
All CD14$^+$	0.037	decrease

* - One-tailed p-value. Sample size of 15 patients using Wilcoxon rank statistic.

5

These data show that administration of WF10 *in vivo* to humans shows an increase in the production of CD28$^-$ subset of CD8$^+$ T-cells. The data also shows an increase in macrophage activation leading to phagocytosis. The data also shows no evidence of RBC hemolysis. When 10 coupled with the *in vitro* studies showing the inhibition of antigen presentation for CD4$^+$ cells, it is believed that administration of WF-10 *in vivo* will result in inhibition and/or prevention of antigen presentation in APC, as well as stimulate macrophage activation resulting in increased phagocytosis.

15

Example 6

Based on the *in vivo* data above, administration of WF10 has shown a consistent down regulation of CD14$^+$/DR$^+$ cells achieving statistical 20 significance. In addition, WF10 administration *in vivo* has shown overall reduction of CD3$^+$/CD8$^+$/CD28$^+$ cells, and significant increased levels of CD3$^+$/CD8$^+$/CD28$^-$ cells of long-term duration. These cells are believed by the inventors to be responsible for antigen specific immune tolerance by

producing clonal anergy. The *in vitro* data above also shows that WF10 is effective in inhibiting and/or preventing antigen presentation and producing clonal anergy. This reduced antigen presentation may be critical in B-cell lymphoma and thus, WF10 therapy may be of benefit. One case history
5 with B-cell lymphoma responded to WF10 therapy with a notable reduction of tumor size with no reoccurrence to date.

Adult patients having low grade follicular lymphoma are selected based on their lack of enrollment in current therapy regimens. Fifteen patients having lymph nodes > 1 cm in diameter at baseline confirmed by
10 CT scan will be enrolled in an open-label, single arm, single center study. Patients will receive periodic 0.5 ml/kg infusions of WF10 from days 1-5 (week 1) and days 8-12 (week 2). After screening evaluations are completed (about 14 days), eligible patients will attend pre-study visit in week 0 to acquire the baseline data.

15 Screening criteria include the following:

male or female patients greater than 18 years of age;

histologically confirmed follicular lymphoma;

measurable disease defined as having lymph nodes > 1 cm in diameter as measured by CT;

20 adequate renal function documented by a serum creatinine < 2 times in institution's ULN;

adequate liver function documented by a serum billrubin less than or equal to 1.5 mg/dl and SGOT (AST) or SGPT (ALT) < 5 times the institutional upper limit of normal;

25 written informed consent to participate in this study and a willingness to comply with all procedures and scheduled visits;

hemoglobin > 9.0 g/dl for woman and > 10.0 g/dl for men;

platelet count > 75,000/mm^2; and

absolute neutrophil count > 750/mm^2.

WF10 will be applied at a dose of 0.5 ml per kg of body weight diluted into 250 to 500 ml normal saline administered by intravenous infusion of 1 hour duration. CT measurements will be taken to determine tumor size at week 0, on day 15, day 30 and day 45. Follow-up period will
5 last for a duration of 3 months with final CT measurements on day 90.

CT measurements will reveal that administration of WF10 results in a reduction of lymph node size. Patients also will exhibit an increase in $CD3^+/CD8^+/CD28^-$, an increase in $CD14^+/DR^+$ and an increase in CD40 T cell subsets.

10 While the invention has been described in detail with reference to the examples and particularly preferred embodiments, those skilled in the art will appreciate that various modifications can be made to the invention without departing from the spirit and scope thereof. All documents referred to above are incorporated by reference. In particular, the PCT patent
15 application WO 99/17787 is incorporated by reference in its entirety herein.

APPENDIX A: NEW CLAIMS

1. A method of treating cancer in a mammal, wherein said cancer is characterized by a reduced expression of the DCC protein, comprising administering an aqueous solution comprising a stabilized chlorite solution.

2. The method of claim 1, wherein the cancer is selected from the group consisting of colon carcinoma, gastric carcinoma, esophageal carcinoma, rectal carcinoma, pancreatic carcinoma, prostate carcinoma, glioma and neuroblastoma.

3. The method of claim 2, wherein said cancer is colon carcinoma.

4. The method of claim 2, wherein said cancer is gastric carcinoma.

5. The method of claim 2, wherein said cancer is esophageal carcinoma.

6. The method of claim 2, wherein said cancer is rectal carcinoma.

7. The method of claim 2, wherein said cancer is pancreatic carcinoma.

8. The method of claim 2, wherein said cancer is prostate carcinoma.

9. The method of claim 2, wherein said cancer is glioma.

10. The method of claim 2, wherein said cancer is neuroblastoma.

11. A method of treating cancer in a mammal comprising:

(a) removing macrophages from the mammal;

(b) contacting the macrophages with an effective amount of an aqueous solution comprising a stabilized chlorite solution under conditions sufficient to increase the expression of DCC in the macrophages; and

(c) introducing the macrophages from step (b) back into the mammal.

12. The method of claim 11, wherein said cancer is characterized by a reduced expression of the DCC protein.

13. The method of claim 12, wherein the cancer is selected from the group consisting of colon carcinoma, gastric carcinoma, esophageal carcinoma, rectal carcinoma, pancreatic carcinoma, prostate carcinoma, glioma and neuroblastoma.

14. The method of claim 13, wherein said cancer is colon carcinoma.

15. The method of claim 13, wherein said cancer is gastric carcinoma.

16. The method of claim 13, wherein said cancer is esophageal carcinoma.

17. The method of claim 13, wherein said cancer is rectal carcinoma.

18. The method of claim 13, wherein said cancer is pancreatic carcinoma.

19. The method of claim 13, wherein said cancer is prostate carcinoma.

20. The method of claim 13, wherein said cancer is glioma.

21. The method of claim 13, wherein said cancer is neuroblastoma.