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(72) Inventors; and
(71) Applicants : **ONG, Christopher John** [CA/CA]; #906 - 1189 Howe Street, Vancouver, British Columbia V6Z 2X4, CANADA (CA). **OGLOFF, Nadya** [CA/CA]; 306 - 8775 Oster Street, Vancouver, British Columbia V6P 4E8 (CA).

(74) Agent: **SMART & BIGGAR**; Box 11560, Vancouver Centre, 2200 - 650 West Georgia Street, Vancouver, British Columbia V6B 4N8 (CA).

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(54) Title: AMINOACYL TRNA-SYNTHETASE INHIBITORS AS BROAD-SPECTRUM IMMUNOSUPPRESSIVE AGENTS

(57) Abstract: Autoimmune disorders, transplant rejection, and inflammatory disorders share a common origin in that the host's immune system is activated such that it attacks otherwise healthy tissue. Aminoacyl t-RNA synthetase inhibitors, such as borrelidin, have now been shown to act as broad spectrum immunosuppressants. Said compounds, and compositions comprising said compounds, are useful in the treatment of the aforementioned diseases.

AMINOACYL tRNA-SYNTHETASE INHIBITORS AS BROAD-SPECTRUM IMMUNOSUPPRESSIVE AGENTS

TECHNICAL FIELD

The present invention relates to the fields of inflammation and immunologic diseases.

BACKGROUND OF THE INVENTION

Autoimmune diseases and inflammation are conditions triggered by aberrant reactions of the human immune system. In autoimmunity, the patient's immune system is activated against the body's own proteins. In inflammatory conditions, it is the overreaction of the immune system, and its subsequent downstream signaling, which causes problems. Transplant rejection occurs when a transplanted organ or tissue fails to be accepted by the body of the transplant recipient. This happens when the immune system of the recipient attacks the transplanted organ or tissue because it recognizes the transplanted organ/tissue as foreign.

Inflammatory Conditions

Inflammation is the body's normal physiological response to injury, infection or foreign substances. The inflammatory response is initiated in response to injury (*e.g.*, and foreign particles) and infection (*e.g.*, bacterial or viral infection) by multiple events, including chemical mediators (*e.g.*, cytokines and prostaglandins) and inflammatory cells (*e.g.*, leukocytes). Clinically, inflammation is characterized by increased blood flow to the tissue, causing pyrexia, erythema, induration, and pain.

Although the ability to mount an inflammatory response is essential for survival, the ability to control inflammation is also necessary for health. The response requires a well-balanced interplay between the humoral and cellular immune elements of the adaptive immune response to eliminate harmful agents and allow for the initiation of repair of damaged tissue. When this delicately balanced interplay is disrupted, the inflammatory response may result in considerable tissue damage and altering the function of affected tissue often causing more harm than the original insult that initiated the reaction. Inflammatory diseases are a group of disorders that are characterized by uncontrolled or excessive inflammatory responses and clinical intervention is needed to prevent tissue damage and organ dysfunction. Diseases such as Crohn's disease, asthma,

allergies, septic shock syndrome, atherosclerosis, inflammatory bowel disease among other clinical conditions are characterized by chronic inflammation.

Autoimmune Conditions

A substantial proportion of the population suffers from autoimmune disease conditions, which are often chronic, debilitating, and life-threatening. There are more than 60 identified autoimmune disorders that include rheumatoid arthritis, psoriasis, multiple sclerosis, idiopathic thrombocytopenia purpura, vitiligo, systemic lupus erythematosus, Sjogren's syndrome, myasthenia gravis, and uveitis.

Transplant Rejection

Rejection is the consequence of the recipient's alloimmune response to the nonself antigens expressed by donor tissues.

Following transplantation, T cells are activated by the presence of allogeneic human leukocyte antigen (HLA) molecules. The HLA genes involved in the immune response have been classified into class I and II; both class I and II molecules act to present antigens to T cells, a process that initiates the adaptive immune response.

Allogeneic MHC antigens provoke the strongest immunologic responses. These reactions can cause rapid elimination of donor cells and graft rejection, thus representing the major hurdle to successful allograft engraftment.

T cells recognize the allogeneic HLA molecules either directly or indirectly. In direct presentation, T cells recognize the determinant peptides on the intact HLA molecules displayed on the surface of the transplanted cells. In indirect presentation, donor HLA molecules are processed and presented as peptides by the recipient's antigen-presenting cells (APCs). APCs, which include dendritic cells, macrophages, B cells, and endothelial cells, possess co-stimulatory adhesion molecules that serve as ligands for counterreceptors on T cells. APCs also provide essential co-stimulatory signals that promote T-cell proliferation and differentiation into either helper or effector lymphocytes.

In hyperacute rejection, transplant patients are serologically presensitized to alloantigens (*i.e.*, graft antigens are recognized as nonself). Histologically, numerous polymorphonuclear leukocytes (PMNs) exist within the graft vasculature and are associated with widespread microthrombi formation and platelet accumulation. Little or no leukocyte infiltration occurs. Hyperacute rejection manifests within minutes to hours of

graft implantation but rejection has become relatively rare since the introduction of routine pretransplantation screening of graft recipients for antidonor antibodies.

In acute rejection, graft antigens are recognized by T cells; the resulting cytokine release eventually leads to tissue distortion, vascular insufficiency, and cell destruction. Histologically, leukocytes are present, dominated by equivalent numbers of macrophages and T cells within the interstitium. These processes can occur within 24 hours of transplantation and occur over a period of days to weeks.

In chronic rejection, pathologic tissue remodeling results from peritransplant and posttransplant trauma. Cytokines and tissue growth factor induce smooth muscle cells to proliferate, to migrate, and to produce new matrix material. Interstitial fibroblasts are also induced to produce collagen. Histologically, progressive neointimal formation occurs within large and medium arteries and, to a lesser extent, within veins of the graft. Leukocyte infiltration usually is mild or even absent. All these result in reduced blood flow, with subsequent regional tissue ischemia, fibrosis, and cell death.

Immunosuppression

A number of potent immunosuppressive compounds of microbial origin have been used in the treatment or prevention of transplant rejection, including cyclosporin and the macrolidese, rapamycin and fujimycin. Rapamycin inhibits response to interleukin-2 (IL-2) and thereby blocks activation of T lymphocytes. Cyclosporin is thought to bind to cyclophilin of immunocompetent lymphocytes, including T lymphocytes which inhibits calcineurin thus preventing a transcription of interleukin-2. In contrast, fujimycin inhibits production of IL-2. Synthetic immunosuppressants such as mycophenolate and dexamethasone have also been used for transplant rejection. The latter drug is a glucocorticoid exhibiting a broad-spectrum of anti inflammatory and immunosuppressant activities.

Aminoacyl-t-RNA (aa-RS) synthetase inhibitors are agents that inhibit activity of enzyme(s) that catalyze transfer of amino acids to their cognate tRNA molecules. The largest group of such inhibitors function as reaction-intermediate mimics. Examples are mupirocin as well as sulfamoyladenoisine analogues of aa-AMP, the latter group being a form of "universal" aa-RS synthetase inhibitor. Individual inhibitors of particular aa-RS synthetases are known, such as REP8839 (a selective inhibitor of bacterial Met RS), reveromycin (an inhibitor of human Ile RS) and the macrolide-polyketide, borrelidin

which inhibits Thr-RS. Inhibitors of aa-RS synthetases are noted as having antibacterial and antiproliferative activity. For example, borrelidin is known as an antibacterial agent as well having antiangiogenic and antimetastatic properties.

It has been reported that analogues of aa-AMP bearing different amino acids (based on a sulfamoyladenosine structure) inhibit proliferation of stimulated T-cells (van de Vijver, P. *et al.* (2008) *J. Med. Chem.* 51: 3020-3029).

SUMMARY OF THE INVENTION

This invention is based on the discovery that aa-RS synthetase inhibitors demonstrate broad-spectrum immunosuppressant activity against immune cells of both myeloid and lymphoid origin, thus being capable of suppressing both innate and adaptive immune responses. Potency and selectivity of such inhibitors with regard to immune cells makes such inhibitors useful in modulating a plurality of immune responses. Such agents can be useful in the treatment of inflammatory diseases or conditions having an immune cell component, autoimmune diseases as well as other conditions and diseases having an immune component (such as asthma and allergic responses). Diseases or conditions that would benefit from a multi-prong suppression of different immune cell participants may be treated. Furthermore, since it is now known that inhibitors of aminoacyl-tRNA synthetase enzymes effect both activation and proliferation of various immune cells, such inhibitors show value for minimizing the onset of adverse immunological responses making such agents particularly useful in situations such as prevention of transplant rejection. For example, such inhibitors may be administered prior to or at the same time as transplantation in an effort to reduce activation of the host's T cells by the presence of allogeneic antigens introduced with or expressed by the donor tissue. The ability of such inhibitors to affect B cells, mast cells, macrophages and neutrophils contribute to the broad-spectrum effects of such inhibitors. Reduced effect of such inhibitor on non-immune cells of a similar lineage (fibroblasts) highlight the selective action of such inhibitors.

Various embodiments of the invention provide use of an aminoacyl tRNA synthetase (aa-RS) inhibitor as an inhibitor of immune cells, providing that when the immune cells to be inhibited are solely T-cells and the inhibitor comprises adenine joined

to a sugar molecule as a substituent, the inhibition comprises inhibition of activation of the T-cells.

Various embodiments of the invention provide use of an aminoacyl tRNA synthetase (aa-RS) inhibitor for preparation of a medicament for use as an inhibitor providing that when the immune cells to be inhibited are solely T-cells and the inhibitor comprises adenine joined to a sugar molecule as a substituent, the inhibition comprises inhibition of activation of the T-cells.

Various embodiments of the invention provide a pharmaceutical composition for use as an inhibitor of immune cells, wherein the composition comprises a pharmaceutically acceptable carrier or excipient and an aminoacyl tRNA synthetase (aa-RS) inhibitor providing that when the immune cells to be inhibited are solely T-cells and the inhibitor comprises adenine joined to a sugar molecule as a substituent, the inhibition comprises inhibition of activation of the T-cells.

Various embodiments of the invention provide a pharmaceutical formulation for use as an inhibitor of immune cells, wherein the formulation is in oral, topical, parenteral or inhalation form and comprises an aminoacyl tRNA synthetase (aa-RS) inhibitor providing that when the immune cells to be inhibited are solely T-cells and the inhibitor comprises adenine joined to a sugar molecule as a substituent, the inhibition comprises inhibition of activation of the T-cells.

Various embodiments of the invention a method for treatment or prevention of an inflammatory condition or suppression of immune cells in a subject in need thereof, wherein the method comprises administering to the subject an effective amount of an amino acid tRNA synthetase (aa-RS) inhibitor, providing that when immune cells to be inhibited are solely T-cells and the inhibitor comprises adenine joined to a sugar molecule as a substituent, the inhibition comprises inhibition of activation of the T-cells.

In accordance with one embodiment, there is provided a method of treating inflammation in a subject. The method involves administering to the subject an effective dose of an aminoacyl tRNA synthetase inhibitor. The method may involve treating inflammation which includes an adaptive immune response or an innate immune response. The method may further involve treating inflammation which is associated with an autoimmune disease. The method may further involve treating inflammation which includes the activation of any one of the following cell types: a T cell, a B cell, a macrophage, a mast cell, a neutrophil, or a monocyte. The method may further involve

treating inflammation which includes the proliferation of any one of the following cell types: a B cell, a macrophage, a mast cell, a neutrophil, or a monocyte. The method may further involve treating inflammation which is not associated with rheumatoid arthritis or psoriasis. The method may involve treating inflammation with, for example, a threonyl tRNA synthetase inhibitor, or a borrelidin compound or borrelidin.

In accordance with another embodiment, there is provided an aminoacyl tRNA synthetase inhibitor for treating inflammation. The inhibitor may be used to treat inflammation which includes an adaptive immune response or an innate immune response, or inflammation which is associated with an autoimmune disease, or inflammation which includes the activation of any one of the following cell types: a T cell, a B cell, a macrophage, a mast cell, a neutrophil, or a monocyte. The inhibitor may be used to treat inflammation which includes the proliferation of any one of the following cell types: a B cell, a macrophage, a mast cell, a neutrophil, or a monocyte. The inhibitor may be used to treat inflammation which is not associated with rheumatoid arthritis or psoriasis. The inhibitor may include, for example, a threonyl tRNA synthetase inhibitor, a borrelidin compound or borrelidin.

In accordance with another embodiment, there is provided a pharmaceutical composition for treating inflammation. The composition includes an aminoacyl tRNA synthetase inhibitor and a pharmaceutically acceptable carrier. The composition may be used to treat inflammation which includes an adaptive immune response or an innate immune response, inflammation which is associated with an autoimmune disease, or inflammation which includes the activation of any one of the following cell types: a T cell, a B cell, a macrophage, a mast cell, a neutrophil, or a monocyte. The composition may be used to treat inflammation which includes the proliferation of any one of the following cell types: a B cell, a macrophage, a mast cell, a neutrophil, or a monocyte. The composition may be used to treat inflammation which is not associated with rheumatoid arthritis or psoriasis. The composition may include, for example, a threonyl tRNA synthetase inhibitor, or a borrelidin compound or borrelidin.

In accordance with another embodiment, there is provided a use for an aminoacyl tRNA synthetase inhibitor for treating inflammation. In accordance with another aspect of the invention, there is provided a use for a aminoacyl tRNA synthetase inhibitor in the manufacture of a medicament for treating inflammation. The uses may involve treating inflammation which includes an adaptive immune response or an innate immune response.

Further, the uses may involve treating inflammation which is associated with an autoimmune disease. The uses provided herein may also involve treating inflammation with the inhibitor or medicament which includes the activation of any one of the following cell types: a T cell, a B cell, a macrophage, a mast cell, a neutrophil, or a monocyte. Further, the uses may involve treating inflammation which includes the proliferation of any one of the following cell types: a B cell, a macrophage, a mast cell, a neutrophil, or a monocyte. Further, the uses may involve treating inflammation which is not associated with rheumatoid arthritis or psoriasis. The uses may involve, for example, the use of threonyl tRNA synthetase inhibitors, or borrelidin compound or borrelidin.

In accordance with another aspect of the invention, a method is described that uses borrelidin alone or in combination with other medication or intervention as an immunosuppressive drug during organ transplantation.

In accordance with another aspect of the invention, there is provided a kit for detecting tumor necrosis factor (TNF) in a sample, the kit comprising at least one antibody specific for TNF.

In accordance with another aspect of the invention there is provided a method for detecting B and T cell proliferation in a subject, the method comprising the use of 3H-Thymidine incorporation into dividing cells as a measure of T or B cell proliferation.

This invention also provides use of aminoacyl tRNA synthetase inhibitors for *in vitro* application in the inhibition of the immune cells described herein.

This invention also contemplates *ex vivo* treatment of immune cells which are intended for introduction into a subject.

The present invention is based, in part, on the discovery that aminoacyl tRNA synthetase inhibitors are able to inhibit B cell proliferation and function, macrophage function, and T cell function.

In accordance with one aspect of the invention, a method is described that inhibits B cell proliferation in a subject having a B-cell dependent autoimmune disease condition, through inhibiting aminoacyl tRNA synthetase activity. Such compound may be selected from a group of aminoacyl tRNA synthetase inhibitors.

In accordance with another aspect of the invention, a method is described that uses aminoacyl tRNA synthetase alone or in combination with other medication or intervention to treat B cell dependent autoimmune diseases.

In accordance with yet another aspect of the invention, a method is described that inhibits macrophage proliferation in a subject having an inflammatory disease condition, through inhibiting aminoacyl tRNA synthetase activity. Such compound may be selected from a group of aminoacyl tRNA synthetase inhibitors.

In accordance with another aspect of the invention, a method is described that uses aminoacyl tRNA synthetase inhibitors alone or in combination with other medication or intervention to treat inflammatory diseases.

In accordance with yet another aspect of the invention, a method is described that inhibits T cell proliferation and/or activation in a subject having an organ transplantation, through inhibiting aminoacyl tRNA synthetase activity. Such compound may be selected from a group of aminoacyl tRNA synthetase inhibitors.

In accordance with another aspect of the invention, a method is described that uses aminoacyl tRNA synthetase inhibitors alone or in combination with other medication or intervention as an immunosuppressive drug during organ transplantation.

In accordance with another aspect of the invention, there is provided a kit for detecting tumor necrosis factor (TNF) in a sample, the kit comprising at least one antibody specific for TNF.

In accordance with another aspect of the invention there is provided a method for detecting B and T cell proliferation in a subject, the method comprising the use of 3H-Thymidine incorporation into dividing cells as a measure of T or B cell proliferation.

Other aspects and features of the present invention would become apparent to those ordinarily skilled in the art by reviewing the following description of specific embodiments of the invention in conjunction with accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

In drawings which illustrate embodiments of the invention,

Figure 1 is a graph showing the effect of borrelidin on B-cell proliferation.

Figure 2 is a graph showing the effect of borrelidin on T-cell proliferation.

Figure 3 is graph showing the effect of borrelidin on fibroblasts.

Figure 4 is a graph showing the effect of borrelidin on the production of cytokines by T-cells upon stimulation.

Figures 5a and 5b show the expression of CD25 and CD69 in borrelidin-treated CD4-positive T-cells.

Figure 6 is a graph showing the effect of L-threonine on cell growth in borrelidin treated primary T-cells.

Figure 7 is a photograph of an immunoblot for the C/EBP homologous protein (CHOP) protein.

Figure 8 is graph showing the effect of borrelidin on LPS-induced TNFα levels in splenocytes.

Figures 9a and 9b are graphs showing the effect of borrelidin on the translation of TNF-α in primary macrophages.

Figure 10 is a graph showing the effect of borrelidin on lipopolysaccharide-induced cytokine production in primary mouse splenocytes.

Figure 11 is a graph showing the effect of borrelidin on apoptosis in macrophages purified from splenocytes.

Figure 12 is a graph showing the effect of borrelidin on Annexin V expression and caspase 3 activation in macrophages purified from splenocytes.

Figures 13a and 13b are graphs showing the effect of borrelidin on mRNA transcripts for TNFα and CHOP in primary macrophages.

Figure 14 is a graph showing the effect of borrelidin on TNF-α-release from anti-IgE murine bone marrow derived mast cells.

Figure 15 is a graph showing the effect of borrelidin on myeloperoxidase (MPO) levels *in vivo*.

Figure 16 is a graph showing the effect of borrelidin on neutrophil recruitment into the conjunctiva.

DETAILED DESCRIPTION

Any terms not directly defined herein shall be understood to have the meanings commonly associated with them as understood within the art of the invention. Certain terms are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions, devices, methods and the like of embodiments of the invention, and how to make or use them. It will be appreciated that the same thing may be said in more than one way. Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein. No significance

is to be placed upon whether or not a term is elaborated or discussed herein. Some synonyms or substitutable methods, materials and the like are provided. Recital of one or a few synonyms or equivalents does not exclude use of other synonyms or equivalents, unless it is explicitly stated. Use of examples in the specification, including examples of terms, is for illustrative purposes only and does not limit the scope and meaning of the embodiments of the invention herein.

In accordance with another aspect of the invention, a method is described that uses borrelidin alone or in combination with other medication or intervention as an immunosuppressive drug during organ transplantation.

In accordance with another aspect of the invention, there is provided a kit for detecting tumor necrosis factor (TNF) in a sample, the kit comprising at least one antibody specific for TNF.

In accordance with another aspect of the invention there is provided a method for detecting B and T cell proliferation in a subject, the method comprising the use of 3H-Thymidine incorporation into dividing cells as a measure of T or B cell proliferation.

This invention also provides use of aminoacyl tRNA synthetase inhibitors for *in vitro* application in the inhibition of the immune cells described herein.

This invention also contemplates *ex vivo* treatment of immune cells which are intended for introduction into a subject.

The present invention is based, in part, on the discovery that an aminoacyl tRNA synthetase inhibitors is able to inhibit B cell proliferation and function, macrophage function, and T cell function.

In accordance with one aspect of the invention, a method is described that inhibits B cell proliferation in a subject having a B-cell dependent autoimmune disease condition, through inhibiting aminoacyl tRNA synthetase activity. Such compound may be selected from a group of aminoacyl tRNA synthetase inhibitors.

In accordance with another aspect of the invention, a method is described that uses aminoacyl tRNA synthetase alone or in combination with other medication or intervention to treat B cell dependent autoimmune diseases.

In accordance with yet another aspect of the invention, a method is described that inhibits macrophage proliferation in a subject having an inflammatory disease condition, through inhibiting aminoacyl tRNA synthetase activity. Such compound may be selected from a group of aminoacyl tRNA synthetase inhibitors.

In accordance with another aspect of the invention, a method is described that uses aminoacyl tRNA synthetase inhibitors alone or in combination with other medication or intervention to treat inflammatory diseases.

In accordance with yet another aspect of the invention, a method is described that inhibits T cell proliferation and/or activation in a subject having an organ transplantation, through inhibiting aminoacyl tRNA synthetase activity. Such compound may be selected from a group of aminoacyl tRNA synthetase inhibitors.

In accordance with another aspect of the invention, a method is described that uses aminoacyl tRNA synthetase inhibitors alone or in combination with other medication or intervention as an immunosuppressive drug during organ transplantation.

In accordance with another aspect of the invention, there is provided a kit for detecting tumor necrosis factor (TNF) in a sample, the kit comprising at least one antibody specific for TNF.

In accordance with another aspect of the invention there is provided a method for detecting B and T cell proliferation in a subject, the method comprising the use of 3H-Thymidine incorporation into dividing cells as a measure of T or B cell proliferation.

As used herein, "aminoacyl tRNA synthetase" refers to an enzyme that catalyzes the transfer of an amino acid to its cognate tRNA as described below:

$$ATP + amino\ acid + cognate\ tRNA \rightarrow AMP + diphosphate + aminoacyl\text{-}tRNA$$

Briefly, the aminoacyl tRNA synthetase first binds ATP and the corresponding amino acid or its precursor to form an aminoacyl-adenylate and release inorganic pyrophosphate (PP_i). The adenylate-aaRS complex then binds the appropriate tRNA molecule, and the amino acid is transferred from the aa-AMP to either the 2'- or 3'-OH of the last tRNA base (A76) at the 3'-end. Some synthetases also mediate a proofreading reaction to ensure high fidelity of tRNA charging; if the tRNA is found to be improperly charged, the aminoacyl-tRNA bond is hydrolyzed.

As used herein, the term "aminoacyl tRNA synthetase inhibitor" refers to inhibitors of the reaction involving aminoacyl tRNA synthetase as defined above.

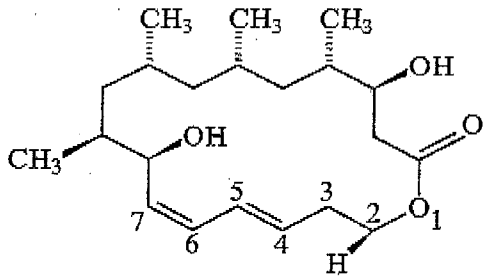
As used herein, a "threonyl tRNA synthetase" (also referred to herein as Thr-RS) is an enzyme that catalyzes the following chemical reaction (aminoacylation):

$$ATP + L\text{-}threonine + tRNAThr \rightarrow AMP + diphosphate + L\text{-}threonyl\text{-}tRNAThr$$

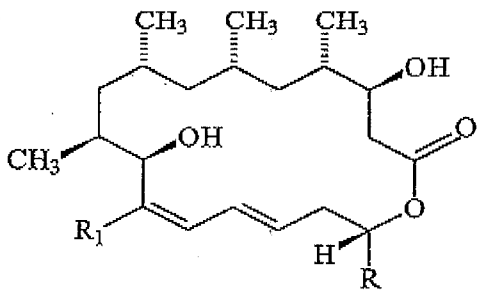
Threonyl tRNA synthetase is a member of a larger family of aminoacyl tRNA synthetases, defined above.

As used herein, "threonyl tRNA synthetase inhibitor" refers to inhibitors of threonyl tRNA synthetase, described above, that inhibit the reaction shown above by binding to the enzyme and preventing threonine from forming L-threonyl-tRNAThr. The inhibitory activity can be tested by standard assays such as that discussed in Beyer *et al.*, (2008) "Methods to Assay Inhibitors of tRNA Synthetase Activity" in Methods in Molecular Medicine, Vol. 142: New Antibiotic Targets (Ed. Champney), Humana Press Inc., Totowa, NJ. Briefly, this assay measures the amount of [14C] radiolabelled threonine that is conjugated to uncharged tRNA. [14C]-Aminoacyl-tRNA is separated from [14C] amino acid by ethanol precipitation followed by filtration through glass fibre filters. Filter-bound radioactivity is detected with a scintillation counter.

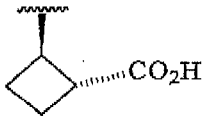
As used herein, the term "borrelidin compound" are compounds (which include the specific compound known as borrelidin) that inhibit threonyl tRNA synthetase and have the following core structure. A variety of substituent groups are present at C2 and C7.



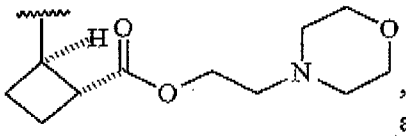
A subset of borrelidin compounds has the following structure.



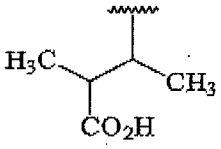
Methods and synthesis of borrelidin compounds are known in the art. Examples of R groups include:



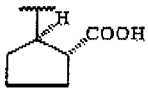
, where R_1 is CN (as described in WO2007/135078);



, where R_1 is CN (as described in WO2007/135078); and



, where R_1 is CN or CO_2H (as described in WO2004/058976).

The specific compound known as borrelidin has an R_1 of CN and an R group of  and is commercially available from numerous commercial sources including Alexis Biochemicals and AG Scientific.

As used herein, "amino acid" refers to natural amino acids, unnatural amino acids, and amino acid analogs, all in their D and L stereoisomeric forms. Natural amino acids include alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, and tyrosine. Unnatural amino acids include, but are not limited to azetidinecarboxylic acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, 2,4 diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, norvaline, norleucine, ornithine and pipecolic acid. Amino acid analogs include the natural and unnatural amino acids which are chemically blocked, reversibly or irreversibly, or modified on their N-terminal amino group or their side-chain groups, as for example, methionine sulfoxide, methionine sulfone, S-(carboxymethyl)-cysteine, S-(carboxymethyl)-cysteine sulfoxide and S-(carboxymethyl)-cysteine sulfone.

As used herein a "subject" refers to an animal, such as a mammal. Specific animals include a rodent such as a rat, mouse or rabbit. A subject may further be a human, alternately referred to as a patient. A subject may further be a transgenic animal.

As used herein "antigen" refers to a protein molecule that often protrudes from the surface of a cell that can induce an immune response. The epitope is the part of the molecule that is responsible for this feature, whereas the carrier molecule results in the activity as an antigen.

As used herein "major histocompatibility complex" (MHC) refers to a set of genes that encode cell-surface antigen-presenting proteins. At maturity, MHC molecules are anchored in the cell membrane, where they display short polypeptides to T cells, via the T cell receptors (TCRs). The polypeptides may be "self", that is, originating from a protein created by the organism itself, or they may be foreign ("non-self"), originating from bacteria, viruses, pollen, etc. The overarching design of the MHC-TCR interaction is that T cells should ignore self peptides while reacting appropriately to the foreign peptides.

As used herein, the term "allogenic" refers to any two individuals from the same species that differ at the MHC, as defined herein. Rejection of grafted tissue from unrelated donors usually results from T-cell responses to allogenic MHC molecules expressed by the grafted tissue.

As used herein, the term "the half maximal effective concentration" (EC50) refers to the concentration of a drug or antibody which induces a response halfway between the baseline and maximum. The EC50 of a graded dose response curve therefore represents the concentration of a compound where 50% of its maximal effect is observed.

As used herein, the term "effective dose" refers to a dose capable of treating a subject for a disorder, such as an immunologic disease as defined herein, including a dose effective to achieve temporary and/or permanent alleviation of some or all of the symptoms associated with such immunologic disease.

As used herein, the term "inflammation" refers to a tissue response to injury, irritation, or infection. Inflammation is typically characterized by pain, redness, swelling, and sometimes loss of function. Inflammation includes, but is not limited to autoimmune inflammation, acute inflammation, catarrhal inflammation, chronic inflammation, exudative inflammation, fibrinous inflammation, granulomatous inflammation, hyperplastic inflammation, interstitial inflammation, parenchymatous inflammation, plastic inflammation, proliferous inflammation, pseudomembranous inflammation,

purulent inflammation, serous inflammation, subacute inflammation, suppurative inflammation, and ulcerative inflammation. Inflammation can also be caused by and/or associated with transplant rejection, wound healing, hypertrophic scarring, burns, chemical irritants, frostbite, toxins, infection by pathogens, physical injury, including injury caused by blunt instruments or penetrating injuries, immune reactions due to hypersensitivity, ionizing radiation, foreign bodies, including splinters and dirt, and ultraviolet irradiation, and skin aging.

Immune cells that are associated with inflammation include, but are not limited to: T cells (CD4, CD8, and NKT), B cells, NK cells, mast cells, macrophages, monocytes, neutrophils, and eosinophils.

As used herein, the term "immunologic disease" refers to a disease caused by abnormal or absent immunologic mechanisms, whether humoral, cell-mediated, or both. Autoimmune disease and inflammatory diseases are types of immunological diseases. Inflammatory diseases are often mediated by responses of the innate immune system *i.e.*, an innate immune response mediated by, for example, mast cells, macrophages, monocytes, neutrophils, eosinophils *etc.*), whereas autoimmune diseases are mediated by a response from the adaptive immune system (*i.e.*, an adaptive immune response mediated by, for example, T and/or B cells). However, some autoimmune diseases also have an inflammatory component. Immunosuppression typically refers to suppression of the adaptive immunity *i.e.*, suppression of T and B cells whereas anti-inflammatory agents inhibit innate immunity *i.e.*, macrophages, mast cells *etc.*

Examples of immunologic diseases include but are not limited to the following: Achlorhydra Autoimmune Active Chronic Hepatitis, Acute disseminated encephalomyelitis (ADEM), Addison's disease, Allergic Ocular Conjuctivitis, Alopecia areata, Alzheimer's, Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's Disease), Ankylosing spondylitis, Anti-GBM Nephritis or anti-TBM Nephritis, Antiphospholipid antibody syndrome (APS), Antiphospholipid Syndrome, Aplastic Anemia, Arthritis, Asthma, Atopic Allergy, Atopic Dermatitis, Atopic Keratoconjunctivitis (AKC), Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune Inner Ear Disease (AIED), Autoimmune Lymphoproliferative Syndrome (ALPS), Balo Disease, Behcet's Disease, Berger's Disease (IgA Nephropathy), Bullous pemphigoid, Cardiomyopathy, Celiac Disease, Chagas disease, Chronic Fatigue Immune Dysfunction Syndrome (CFIDS), Chronic obstructive pulmonary disease, Chronic prostatitis, Churg Strauss

Syndrome, Cicatricial Pemphigoid, Cogan's Syndrome, Cold Agglutunin Disease, Colitis, Cranial Arteritis, CREST Syndrome, Crohns Disease (one of two types of idiopathic inflammatory bowel disease "IBD"), Cushing's Syndrome, Dego's Disease, Dermatitis, Dermatomyositis, Devic Disease, Diabetes mellitus type 1, Diabetes, Type 2, Discoid Lupus, Diverticulitis, Dressler's Syndrome, Eczema, Endometriosis, Eosinophilic Fasciitis, Epidermolysis Bullosa Acquisita, Essential Mixed Cryoglobulinemia, Evan's Syndrome, Fibromyalgia, Fibromyositis, Fibrosing Alveolitis, Gastritis, Giant Cell Artertis, Glomerulonephritis, Goodpasture's syndrome, Graft versus host disease, Graves' disease, Guillain-Barré syndrome (GBS), Hashimoto's disease, Hemolytic Anemia, Henoch-Schonlein Purpura, Hepatitis, Hidradenitis suppurativa, Host versus graft, Hughes Syndrome, Hypersensitivities, Idiopathic Adrenal Atrophy, Idiopathic Pulmonary Fibrosis, Idiopathic thrombocytopenic purpura, IgA nephropathy, Inflammatory Demylinating Polyneuropathy, Interstitial cystitis, Irritable Bowel Syndrome, Kawasaki's Disease, Lichen Planus, Lou Gehrig's Disease, Lupoid Hepatitis, Lupus erythematosus, Lyme Disease, Meniere's Disease, Mixed Connective Tissue Disease, Morphea, Multiple Myeloma, Multiple sclerosis (MS), Myasthenia Gravis, Myositis, Narcolepsy, Nephritis, Neuromyotonia, Ocular Cicatricial Pemphigoid, Osteoarthritis, Osteoporosis, Parkinsons, Pars Planitis, Pemphigus Vulgaris, Pelvic Inflammatory Diseases, Pernicious anaemia, Polyglandular Autoimmune Syndromes, Polymyalgia Rheumatica (PMR), Polymyositis, Primary biliary cirrhosis, Primary Sclerosing Cholangitis, Psoriatic Arthritis, Raynaud's Phenomenon, Reiter's Syndrome, Reperfusion injury, Rheumatic Fever, Rhinitis, Sarcoidosis, Schizophrenia, Scleritis, Scleroderma, Septic Shock, Sjogren's Syndrome, Sticky Blood Syndrome, Stiff person syndrome, Still's Disease, Sydenham Chorea, Systemic Lupus Erythmatosis (SLE), Takayasu's Arteritis, Temporal arteritis (also known as "giant cell arteritis"), Transplant rejection, Ulcerative Colitis (one of two types of idiopathic inflammatory bowel disease "IBD"), Vasculitis, Vernal Keratoconjunctivitis (VKC), Vitiligo, Wegener's Granulomatosis, and Wilson's Syndrome.

Many immunologic diseases detailed herein are characterized by adverse effects of T cells, or B cells, or neutrophils, or mast cells, or eosinophils, or macrophages, or monocytes. A person skilled in the art would appreciate that various immunologic diseases, as defined herein, are typified by adverse effects of the innate immune system and/or adaptive immune system, and constituent cells thereof. For example, those skilled in the art would appreciate that mast cell activity is associated with atopic diseases

such as asthma, allergies, and allergic ocular conjunctivitis. Serving as a further example, those skilled in the art would appreciate that macrophage activity is associated with autoimmune diseases, chronic inflammation, chronic prostatitis, glomerulonephritis, hypersensitivities, inflammatory bowel disease, pelvic inflammatory disease, reperfusion injury, rheumatoid arthritis, transplant rejection, and vasculitis. Additionally, inflammation mediated in whole or in part by macrophages can be caused by radiation treatment, burns, sunburns, UV-exposure, and physical injury.

As used herein, routes of "administering" agents of the invention may vary, and may for example include, but are not limited to, oral, topical, intradermal, transdermal, parenteral, intravenous, intramuscular, intranasal, subcutaneous, regional, percutaneous, intratracheal, intraperitoneal, intraarterial, intravesical, intratumoral, inhalation, perfusion, lavage, direct injection, and oral administration and formulation. Different ways of preparing an agent for inhalation are known, (US5,875,776; US5,277,175; US6,325,062 and *Remington, infra*). Different ways for preparing an agent for topical administration are known (US5,719,197; US6,562,363 and *Remington, infra*).

As referred to herein, "pharmaceutically acceptable carrier" refers to a diluent, adjuvant, excipient or carrier with which an aminoacyl tRNA synthetase inhibitor is administered.

The aminoacyl tRNA synthetase inhibitors of this invention may optionally be combined with one and other for a synergistic effect and/or combined with other immunosuppressants and/or agents that affect the immune system for further synergistic effects.

Inhibition of an immune cell refers to the inhibition of one or more of the following: (i) the inhibition of an immune cell's motility, (ii) the inhibition of an immune cell's biological activity, including but not limited to an immune cell's activation; or (iii) the inhibition of an immune cell's proliferation.

In another aspect of the invention, it has been demonstrated herein that borrelidin at nanomolar doses is effective in inhibiting B cell proliferation, indicating usefulness in treating B-cell dependent autoimmune diseases or other inflammation that is mediated by B cell proliferation. Further, it has been demonstrated herein that borrelidin inhibits TNFα production following LPS stimulation of macrophages/monocytes, therefore indicating usefulness as an anti- inflammatory agent for the treatment of inflammatory diseases (*e.g.*, colitis, asthma, allergy) that involve macrophages/monocytes.

Further, it has been demonstrated here that borrelidin inhibits T cell proliferation and activation therefore indicating its potential use as an immunosuppressant (*e.g.*, in bone marrow or organ transplantation)

It is further demonstrated herein that borrelidin exerts its immunosuppressive effects via inhibiting pro-inflammatory mediators via the GCN2 pathway. GCN2, a stress-activated eIF2a kinase regulated indole 2,3-dioxygenase (IDO), an inducible intracellular enzyme which catabolises tryptophan creating nutrient starvation conditions (Munn, D.H. *et al.* (2005) *Immunity*, 22:633-64). IDO expressing antigen presenting cells (APC) activate the GCN2 pathway in T-cells, ultimately leading to inhibition of protein translation.

Borrelidin selectively inhibits eukaryotic threonine tRNA synthetase, which triggers GCN with the accumulation of uncharged transfer RNA (Munn, D.H. *et al.* (2005) *Immunity*, 22:633-64).

The phosphorylation of eIF2a inhibits general protein synthesis reducing levels of eIF2-GTP that are required for binding of initiator tRNA to the ribosome (Horner BM *et al.*, 2008). Thus as a result, the overall level of protein translation is decreased.

As shown herein, borrelidin leads to suppression of immune cell activation through upregulation of uncharged tRNA that in turn leads to activation of GCN2 kinase that in turn phosphorylates eIF2a that leads to global inhibition of protein translation while upregulating CHOP and the integrated stress response. All amino acyl tRNA synthetase inhibitors will lead to an increase in uncharged tRNA that can activate the GCN2 pathway.

When immune cells treated with borrelidin are activated by an autoimmune or allergic response, the inventors hypothesized that the inflammatory response will be dampened due to the suppression of protein translation and hence reduce the release of pro-inflammatory mediators. As shown in Figure 5, borrelidin inhibited T cell proliferation via induction of genes in the GCN2 pathway, demonstrating its potential use as an immunosuppressive agent. Compounds as described herein may be in the free form or in the form of a salt thereof. In some embodiments, compounds as described herein may be in the form of a pharmaceutically acceptable salt, which are known in the art (Berge *et al.* (1977) *J. Pharm. Sci.* 66: 1-19. Pharmaceutically acceptable salt as used herein includes, for example, salts that have the desired pharmacological activity of the parent compound (salts which retain the biological effectiveness and/or properties of the parent compound and which are not biologically and/or otherwise undesirable). Compounds as

described herein having one or more functional groups capable of forming a salt may be, for example, formed as a pharmaceutically acceptable salt. Compounds containing one or more basic functional groups may be capable of forming a pharmaceutically acceptable salt with, for example, a pharmaceutically acceptable organic or inorganic acid. Pharmaceutically acceptable salts may be derived from, for example, and without limitation, acetic acid, adipic acid, alginic acid, aspartic acid, ascorbic acid, benzoic acid, benzenesulfonic acid, butyric acid, cinnamic acid, citric acid, camphoric acid, camphorsulfonic acid, cyclopentanepropionic acid, diethylacetic acid, digluconic acid, dodecylsulfonic acid, ethanesulfonic acid, formic acid, fumaric acid, glucoheptanoic acid, gluconic acid, glycerophosphoric acid, glycolic acid, hemisulfonic acid, heptanoic acid, hexanoic acid, hydrochloric acid, hydrobromic acid, hydriodic acid, 2-hydroxyethanesulfonic acid, isonicotinic acid, lactic acid, malic acid, maleic acid, malonic acid, mandelic acid, methanesulfonic acid, 2-napthalenesulfonic acid, naphthalenedisulphonic acid, p-toluenesulfonic acid, nicotinic acid, nitric acid, oxalic acid, pamoic acid, pectinic acid, 3-phenylpropionic acid, phosphoric acid, picric acid, pimelic acid, pivalic acid, propionic acid, pyruvic acid, salicylic acid, succinic acid, sulfuric acid, sulfamic acid, tartaric acid, thiocyanic acid or undecanoic acid. Compounds containing one or more acidic functional groups may be capable of forming pharmaceutically acceptable salts with a pharmaceutically acceptable base, for example, and without limitation, inorganic bases based on alkaline metals or alkaline earth metals or organic bases such as primary amine compounds, secondary amine compounds, tertiary amine compounds, quaternary amine compounds, substituted amines, naturally occurring substituted amines, cyclic amines or basic ion-exchange resins. Pharmaceutically acceptable salts may be derived from, for example, and without limitation, a hydroxide, carbonate, or bicarbonate of a pharmaceutically acceptable metal cation such as ammonium, sodium, potassium, lithium, calcium, magnesium, iron, zinc, copper, manganese or aluminum, ammonia, benzathine, meglumine, methylamine, dimethylamine, trimethylamine, ethylamine, diethylamine, triethylamine, isopropylamine, tripropylamine, tributylamine, ethanolamine, diethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, glucamine, methylglucamine, theobromine, purines, piperazine, piperidine, procaine, N-ethylpiperidine, theobromine, tetramethylammonium compounds, tetraethylammonium

compounds, pyridine, N,N-dimethylaniline, N-methylpiperidine, morpholine, N-methylmorpholine, N-ethylmorpholine, dicyclohexylamine, dibenzylamine, N,N-dibenzylphenethylamine, 1-ephenamine, N,N'-dibenzylethylenediamine or polyamine resins. In some embodiments, compounds as described herein may contain both acidic and basic groups and may be in the form of inner salts or zwitterions, for example, and without limitation, betaines. Salts as described herein may be prepared by conventional processes known to a person skilled in the art, for example, and without limitation, by reacting the free form with an organic acid, an inorganic acid, an organic base or an inorganic base, or by anion exchange or cation exchange from other salts. Those skilled in the art will appreciate that preparation of salts may occur *in situ* during isolation and/or purification of the compounds or preparation of salts may occur by separately reacting an isolated and/or purified compound.

In some embodiments, compounds and all different forms thereof (*e.g.*, free forms, salts, polymorphs, isomeric forms) as described herein may be in the solvent addition form, for example, solvates. Solvates contain either stoichiometric or non-stoichiometric amounts of a solvent in physical association with the compound or salt thereof. The solvent may be, for example, and without limitation, a pharmaceutically acceptable solvent. For example, hydrates are formed when the solvent is water or alcoholates are formed when the solvent is an alcohol.

In some embodiments, compounds and all different forms thereof (*e.g.*, free forms, salts, solvates, isomeric forms) as described herein may include crystalline and/or amorphous forms, for example, polymorphs, pseudopolymorphs, conformational polymorphs, amorphous forms, or a combination thereof. Polymorphs include different crystal packing arrangements of the same elemental composition of a compound. Polymorphs usually have different X-ray diffraction patterns, infrared spectra, melting points, density, hardness, crystal shape, optical and electrical properties, stability and/or solubility. Those skilled in the art will appreciate that various factors including recrystallization solvent, rate of crystallization and storage temperature may cause a single crystal form to dominate.

In some embodiments, pharmaceutical compositions in accordance with this invention may comprise a salt of such a compound, preferably a pharmaceutically or physiologically acceptable salt. Pharmaceutical preparations will typically comprise one or more carriers, excipients or diluents acceptable for the mode of administration of the

preparation, be it by injection, inhalation, topical administration, lavage, or other modes suitable for the selected treatment. Suitable carriers, excipients or diluents include those known in the art for use in such modes of administration.

Suitable pharmaceutical compositions may be formulated by means known in the art and their mode of administration and dose determined by the skilled practitioner. For parenteral administration, a compound may be dissolved in sterile water or saline or a pharmaceutically acceptable vehicle used for administration of non-water soluble compounds such as those used for vitamin K. For enteral administration, the compound may be administered in a tablet, capsule or dissolved in liquid form. The tablet or capsule may be enteric coated, or in a formulation for sustained release. Many suitable formulations are known, including, polymeric or protein microparticles encapsulating a compound to be released, ointments, pastes, gels, hydrogels, or solutions which can be used topically or locally to administer a compound. A sustained release patch or implant may be employed to provide release over a prolonged period of time. Many techniques known to one of skill in the art are described in *Remington: the Science & Practice of Pharmacy* by Alfonso Gennaro, 20th ed., Lippencott Williams & Wilkins, (2000) [*Remington*]. Formulations for parenteral administration may, for example, contain excipients, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

It is understood that it could be potentially beneficial to restrict delivery of the compounds of the invention to the target tissue or cell in which therapeutic effect is desired. Compositions or methods to facilitate localized tissue delivery would be apparent to one of skill in the art.

Compounds or pharmaceutical compositions in accordance with this invention or for use in this invention may be administered by means of a medical device or appliance such as an implant, graft, prosthesis, stent, etc. Also, implants may be devised which are

intended to contain and release such compounds or compositions. An example would be an implant made of a polymeric material adapted to release the compound over a period of time.

An "effective amount" of a pharmaceutical composition according to the invention includes a therapeutically effective amount or a prophylactically effective amount. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as decreased inflammation, increased life span or increased life expectancy. A therapeutically effective amount of a compound may vary according to factors such as the disease state, age, sex, and weight of the subject, and the ability of the compound to elicit a desired response in the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the compound are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as decreased inflammation, increased life span, increased life expectancy or prevention of the progression of an indication.

Typically, a prophylactic dose is used in subjects prior to or at an earlier stage of disease, so that a prophylactically effective amount may be less than a therapeutically effective amount. It is to be noted that dosage values may vary with the severity of the condition to be alleviated. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. The amount of active compound(s) in the composition may vary according to factors such as the disease state, age, sex, and weight of the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It may be advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage.

In some embodiments, compounds and all different forms thereof as described herein may be used, for example, and without limitation, in combination with other treatment methods. For example, compounds and all their different forms as described

herein may be used as neoadjuvant (prior), adjunctive (during), and/or adjuvant (after) therapy in combination with other therapies known to one of ordinary skill in the art.

In general, compounds of the invention should be used without causing substantial toxicity. Toxicity of the compounds of the invention can be determined using standard techniques, for example, by testing in cell cultures or experimental animals and determining the therapeutic index, *i.e.*, the ratio between the LD50 (the dose lethal to 50% of the population) and the LD100 (the dose lethal to 100% of the population). In some circumstances, however, such as in severe disease conditions, it may be necessary to administer substantial excesses of the compositions. Some compounds of this invention may be toxic at some concentrations. Titration studies may be used to determine toxic and non-toxic concentrations. Toxicity may be evaluated by examining a particular compound's or composition's specificity across cell lines. Animal studies may also be used to provide an indication if the compound has any effects on other tissues.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of skill in the art in light of teachings of this invention that changes and modification may be made thereto without departing from the spirit or scope of the invention. All patents, patent applications, and other publications referred to herein are hereby incorporated by reference.

EXAMPLES

Methods and Materials

LPS stimulation of macrophages.

For the analysis of lipopolysaccharide (LPS)-stimulated tumor necrosis factor (TNF) production, spleens were harvested from BALB/C mice and were ground into a single cell suspension followed by purification using a Histopaque column (Sigma). $1x10^6$ splenocytes were seeded per well in RPMI complete with borrelidin for 30 min prior to the addition of 10μg/ml LPS. Supernatants were collected 3 hours post LPS stimulation and TNF determination was performed by enzyme-linked immunosorbent assay (ELISA; BD Biosciences, Mississauga, ON, Canada).

B-cell, T-cell and Fibroblast Proliferation Assays

Proliferation was monitored by [3H]Thymidine incorporation into growing cells. All proliferation assays received an 18 hour overnight pulse of the radioactive thymidine. B-cell Proliferation Assay. Spleens were harvested as described above and seeded $5x10^5$c/well for B-cells in the presence of borrelidin. Cells were then stimulated with 10μg/ml of anti IgM and were allowed proliferation 24 hours prior to a 3H-Thymidine spike. T-Cell Proliferation assay. A 2-way mixed lymphocyte reaction was performed using $2x10^5$ c/well of each Balb/c stimulator and CD1 responder splenocytes. A titration of borrelidin was added to the cells and they were allowed to incubate for 48 hours prior to a 3H-Thymidine spike. Fibroblast Proliferation Assay. Primary fibroblasts were harvested from human foreskin and cultured in KCMSF media (Gibco). Cells were seeded at 3,000 c/well in the presence of borrelidin and allowed to proliferation for 48 hours before the [3H]Thymidine spike.

Borrelidin formulations.

Borrelidin is water soluble. Borrelidin can be formulated, for example, as an eye-drop formulation as a 2% solution in tetrahydrozoline hydrochloride or as a topical formulation as 1:2 DMSO/MeOH.

Mouse acute cutaneous anaphylaxis model.

BALB/C mice aged 6 to 8 weeks were sensitized by cutaneous application of 25μL 0.5% dinitroflourobenzene (DNFB) in acetone to the hairless abdomen of mice for 2 consecutive days. One week from the first application, test substances dissolved in 1:2 DMSO/Methanol were painted on the right ear while the left ear received vehicle control. At 30 minutes after drug application, 0.5% DNFB in 1:4 olive oil:acetone was applied to both ears to induce mast cell degranulation. A 6-mm punch was taken from the ear and immediately frozen in liquid nitrogen for subsequent determination of neutrophil myeloperoxidase (MPO) activity.

Myeloperoxidase (MPO) Assay

Harvested ear tissue was homogenized (Polytron tissue homogenizer) in 1ml of HTAB buffer (0.5g hexadecyltrimethylammonium bromide in 50mM Potassium Phosphate Buffer pH 6.0) for 1 min at 10500 RPM. The lysates were transferred to 1.5ml eppendorf tubes and microfuged 2x at 10,000 RPM, at 4°C for 10 min. Supernatant was

analyzed for MPO activity by the addition of Tetramethyl Benzidine (TMB) and monitoring at 650nm.

Microscopic analysis of splenocytes

Spleens were harvested from Balb/C mice and ground into a single cell suspension. They were then pre-incubated in either Borrelidin (at 50, 5 or 0.5μg/ml) or RPMI complete for 30 min. Cells were then activated for 24 hours using immobilized anti CD3 and anti CD28 antibodies and cell morphology was analysed on a light microscope.

Jurkat treatment and Western Blot Analysis

Jurkat cells were seeded in 1ml RPMI (Roswell Park Medical Institute) medium complete at $1x10^6$ cells per well in a 6 well dish. 24 hours later, borrelidin (5, 10 20ng/ml) or DMSO (dimethyl sulfoxide) were added to each well. Tryptophan depleted media was used as positive control for CHOP, as defined herein up regulation. 24 hours after borrelidin addition, cells were harvested and lysed. 40μg of total lysis extracts were analyzed by a 10% SDS-polyacrylamide gel electrophoresis. The proteins were transferred to the nitrocellulose filter. For immunoblotting the membrane was blocked for 1 hour at room temperature in 5%BSA TBST (Tris-Buffered Saline Tween-20) followed by overnight incubation with anti mouse GAD153 sc-7351 at 1:200 in 1%BSA TBST. After washing 3x with TBST, the membrane was incubated with a Dk anti Mu HRP antibody at 1:2000 for 1 hour at room temperature. The membrane was then wash 3x with TBST and analyzed using ECL.

Western Blot Analysis

10μg of nuclear extract were analyzed by a 10% SDS-polyacrylamide gel electrophoresis. The proteins were transferred to the nitrocellulose filter. For immunoblotting the membrane was blocked for 1 hour at room temperature in 5%BSA TBST followed by overnight incubation with anti mouse GAD153 sc-7351 at 1:500 in 1%BSA TBST. After washing 3x with TBST, the membrane was incubated with a Rb anti Mu HRP antibody at 1:2000 for 1 hour at room temperature. The membrane was then washed 3x with TBST and analyzed using ECL.

The following Examples highlight experiments involving B cells, T cells, macrophages, mast cells, and *in vivo* allergy models.

Example 1. Borrelidin inhibits B-cell proliferation in a dose dependent manner.

The efficacy of borrelidin on inhibiting B cell proliferation was determined by demonstrating its effects on primary mouse splenocytes in vitro. To monitor B cell proliferation splenocytes were stimulated with anti-IgM in the presence or absence of borrelidin. The capacity of B cells to undergo proliferation was measured by 3H-Thymidine incorporation into dividing cells. Splenocytes were harvested from BALB/C mice, treated with borrelidin and then stimulated with 10 μg/ml anti IgM. 24 hours later 3H-Thymidine was spiked into each well at 1uCi/well. Thymidine incorporation was monitored an addition 24 hours later. Proliferation decreased drastically in B cells treated with borrelidin with an EC_{50} of 14.5ng/ml (see Figure 1).

Example 2. Borrelidin inhibits T-cell proliferation in a dose dependent manner

Unprimed T-cells can be activated to proliferate upon being cultured with allogenic stimulator lymphocytes. The activation stimulus is the foreign histocompatibility antigen expressed on the allogenic stimulator cells. The capacity of the T-cells to undergo proliferation can be measured by 3H-Thymidine incorporation into dividing cells. To examine the effect of borrelidin on T cell growth, a two-way mixed lymphocyte reaction (MLR) was performed on BALB/C stimulator and CD1 responder splenocytes. A titration of borrelidin was added to the cells and they were allowed to incubate for 48 hours prior to a 3H-Thymidine spike.

Thymidine incorporation was monitored an addition 24 hours later.

Treatment with borrelidin resulted in a dramatic decrease in T cell proliferation with an EC_{50} of 1.6ng/ml as shown in Figure 2. ΔCPM represents the change in count per minute at each borrelidin concentration between stimulated and unstimulated cells. Interestingly fibroblasts showed a lower sensitivity to borrelidin (Figure 3) than the hematopoietic cells (see Figures 1-2) with an EC_{50} value of 520ng/ml, 50x times less sensitive than the values observed for splenocytes. With respect to the data presented in Figure 3, primary fibroblasts were harvested from human foreskin and maintained in Keratinocyte SFM media. These cells were incubated with borrelidin for 48 hours before 3H-Thymidine was spiked into each well at 1μCi/well.

The morphology of T lymphocytes following treatment with borrelidin was also observed by microscopy. Splenocytes were isolated from BALB/c mice, pre-incubated for 30 minutes in the presence of borrelidin and then stimulated by anti CD3/anti CD28 to

expand the T lymphocytes in culture. Controls contained comparable levels of DMSO. When the stimulated cultures were monitored for morphological differences, there was a clear decrease in proliferation of borrelidin treated cells and also an absence of the traditional blasting characteristics of an activated T cell when compared to the control cells

Example 3. Borrelidin decreases cytokine production by T-cells upon stimulation.

Purified mouse lymphocytes were pretreated with borrelidin (0, 2, 5, 10 ng/ml) or in the absence of tryptophan for 30 minutes prior to stimulation by plate bound anti-CD3/anti-CD28 crosslinking. Supernatants were collected 24 hours later and Il-2, IL-4 and IFN-γ levels were determined by enzyme linked immunosorbant assay (ELISA). Results are shown in Figure 4. Levels of IL-2 are shown in the left-most column; levels of IFN-γ are shown in the middle column; and levels of IL-4 are shown in the right-most column of Figure 4.

Example 4. Borrelidin inhibits expression of T cell activation markers, CD69 and CD25 on CD4 positive T-cells upon stimulation.

Purified mouse lymphocytes were pretreated with borrelidin (0,2,5,10 ng/ml) or in the absence of tryptophan for 30 minutes prior to stimulation by plate bound anti-CD3/anti-CD28 crosslinking. 24 hours later cells were collected and stained using fluorescently labeled antibodies. The CD4 positive population of stimulated lymphocytes was monitored for CD69 and CD25 expression (Figures 5a and 5b).

Example 5. L-threonine rescues inhibition of cell growth in borrelidin treated primary T-cells.

Histopaque purified lymphocytes were harvested from BALB/C and C57Bl/6 mice spleens and added together in the presence of borrelidin for a two-way mixed lymphocyte reaction (MLR). In some wells, 50units/ml of mouse Il-2 or 1mM L-threonine was added to the RPMI media prior to the addition of cells. 48 hours later [^{3}H]-Thymidine was spiked into each well at 1μCi/well. Thymidine incorporation was monitored an addition 18 hours later. ΔCPM represents change in CPM at each borrelidin concentration between stimulated and unstimualted cells (Figure 6).

Example 6. Borrelidin signals through the GCN2 pathway in T-cells.

Nylon wool enriched primary T-cells from purified spleen lymphocytes were stimulated with 50ng/ml PMA and 500ng/ml Ionomycin at $2x10^6$ cells/ml in 4 ml. After 24 hours, either 0, 10 or 20ng/ml borrelidin were added to the cultures. Cell lysates were made in NP40 lysis buffer at 3 hours after borrelidin addition and immunoblots for eIF2α and CHOP were prepared. Both eIF2α and CHOP appeared to be upregulated in the presence of borrelidin.

Example 7. Borrelidin activates CHOP in the GCN2 pathway.

GCN2 is an eIF2a kinase that is activated by stress, and has been previously linked with tumor immunity, autoimmunity and inhibition of protein translation through regulation of indole 2,3-dioxygenase (IDO), an inducible intracellular enzyme which catabolises tryptophan creating nutrient starvation conditions (Munn, D.H. *et al.* (2005) Immunity, 22:633-64). IDO expressing antigen presenting cells (APC) activate the GCN2 pathway in T cells ultimately leading to inhibition of protein translation. To assess whether the effect of borrelidin on T lymphocytes occurred via the GCN2 pathway, Jurkat cells, an immortalized line of T lymphocytes were treated with different dosages of borrelidin or DMSO as a control for 24 hours, and were subjected to western blot analysis for genes involved in the GCN2 pathway. The CHOP gene (also known as gadd153) was used to monitor GCN2 activation in Jurkat cells. CHOP is a well-accepted marker for GCN2 activation and although it can be induced by many other stress conditions, it is known to be induced by amino acid deprivation and requires and intact GCN2 pathway to be expressed. Jurkat cells grown in Tryptophan depleted media was used as a positive control. As shown in Figure 7, the treatment of borrelidin resulted in an increase in CHOP expression in a dose-dependent manner. CHOP was also upregulated when Jurkat cells were cultured in the absence of tryptophan. This data indicates that borrelidin is capable of activating the GCN2 pathway in T lymphocytes thus preventing protein translation, and dampening the immune response during an allergic inflammatory condition or during organ transplantation.

Specific conditions were as follows: Jurkat cells were seeded in 1ml RPMI complete at $1x10^6$ cells per well in a 6 well dish. 24 hours later, borrelidin (5, 10 20ng/ml) or DMSO were added to each well. Tryptophan depleted media was used as positive control for CHOP up regulation. 24 hours after borrelidin addition, cells were harvested,

lysed and subjected to western blot analysis. Briefly, 40μg of total lysis extracts were analyzed by a 10% SDS-polyacrylamide gel electrophoresis. The proteins were transferred to the nitrocellulose filter. For immunoblotting the membrane was blocked for 1 hour at room temperature in 5%BSA TBST followed by overnight incubation with anti mouse GAD153 sc-7351 at 1:200 in 1%BSA TBST. After washing 3x with TBST, the membrane was incubated with a Dk anti Mu HRP antibody at 1:2000 for 1 hour at room temperature. The membrane was then wash 3x with TBST and analyzed using ECL.

Example 8. Borrelidin inhibits macrophage activation in a dose dependent manner.

LPS stimulation of splenocytes is associated with release of pro-inflammatory cytokines such as TNF-α. To examine the effects on borrelidin on LPS stimulated BALB/C mouse splenocytes were pretreated for 30 minutes with a titration of borrelidin prior to stimulation with 10μg/ml of LPS and TNFα levels were determined by ELISA. Borrelidin suppressed TNF- α expression in a dose dependent manner with an EC_{50} of 11ng/ml (see Figure 8). In the absence of LPS induction, there was no TNF- α production.

Example 9. Borrelidin inhibits translation of TNFα in primary macrophages.

Primary macrophages were treated for 1 hour in the presence or absence of GolgiPlug™ (BD) to block gogli export. Macrophages were then treated in the presence (10ng/ml) or absence (0ng/ml) of borrelidin for 30 minutes prior to lipopolysaccaride (LPS) stimulation (0.1μg/ml). At 3 hours supernatnants were collected and cell pellets were lysed useing the non-reducting lysis buffer: 20mM HEPES pH 7.9, 430mM NaCl, 1.5mM MgCl2, 0.2mM EDTA, 25% glycerol. TNFα levels from both supernatants and cell lysates were determined using a TNFα ELISA, as shown in Figures 9a and 9b, respectively. Levels of TNFα in the presence of GolgiPlug™ are shown in the left-most columns for each condition detailed in Figures 9a and 9b. Levels of TNFα in the absence of GolgiPlug™ are shown in the right-most column for each condition detailed in Figures 9a and 9b.

Example 10. Borrelidin inhibits lipopolysaccaride (LPS) induced cytokine/chemokine production in primary mouse splenocytes.

Ficoll- purified splenocytes from Balb/c mice were preincubated in the presence (5.6, 16.7, 50, or 150ng/ml) or absence (0ng/ml) of borrelidin for 30 minutes prior to 0.1μg/ml LPS stimulation. At 3 hours, the supernatants were harvested and

cytokine/chemokine levels were determined using Bio-Plex Mouse Cytokine 23-Plex Panel (BioRad, CA). The results are shown in Figure 10. With reference to Figure 10, the columns represented in Figure 10 for each condition correspond with the following cytokines (from left to right): IL-10, IL-12, MIP-1α, MIP-1β, RANTES, and TNF-α.

Example 11. Borrelidin induces apoptosis as shown by propidum iodine (PI) cell cycle analysis.

Macrophages derived from C57BL/6 splenocytes were preincubated in the presence (2, 5, 10, 20 ng/ml) or absence (0 ng/ml) of borrelidin for 30 minutes prior to 0.1μg/ml LPS stimulation. Twenty-four hours later cells were fixed using ice-cold 80% Ethanol and stained using 50μg/ml PI + 50μg/ml RNase A. The results are shown in Figure 11 where percent apoptosis is based on the percentage of cells in the G_0 population.

Example 12. Borrelidin induces macrophage apoptosis as shown by Annexin V exppression and activation of Caspase 3.

Macrophages derived from C57BL/6 splenocytes were preincubated in the presence (2, 5, 10, 20 ng/ml) or absence (0 ng/ml) of borrelidin for 30 minutes prior to 0.1μg/ml LPS stimulation. 24 hours later cells were collected and separately stained using fluorescently labeled antibodies for Annexin V or activated Caspase 3. The results are shown in Figure 12. With reference to Figure 12, the levels of Annexin V are shown in the left-most column for each condition tested; the levels of activated Caspase 3 are shown in the right-most column for each condition tested.

Example 13. mRNA transcripts for TNFa and CHOP increase in the presence of borrelidin.

Relative quantities (RQ) of TNFα and CHOP in primary macrophages were determined by qPCR based on the conditions described in Figures 13a and 13b. Ubiquitin C and RPL32 were used as endogenous controls, respectively.

Example 14. Borrelidin inhibits TNFa-release from anti-IgE stimulated murine bone marrow derived mast cells (BMMC).

Mature BMMCs were IL-3 starved in IMDM, 10% FCS, 150uM monothioglycerol & Penicillian/Streptomycin for 4 hours at 37°C/5% CO2 followed by a 30 min borrelidin incubation (0, 10, 100, 250, 500, 1000 ng/ml) in IMDM +0.1% BSA.

Cells were stimulated with a final anti IgE Concentration of 1μg/ml for 4 hours. Conditioned media was collected and a TNFα ELISA performed for detection of TNFα levels. Results are shown in Figure 14. With reference to Figure 14, the levels of TNFα associated with 1μg/ml of anti-IgE are shown in the left most column for each condition tested; the levels of TNFα associated with 0μg/ml of anti-IgE are shown in the left most column for each condition tested;

Example 15: Borrelidin inhibits cutaneous anaphylaxis in a mouse allergy model in vivo.

Borrelidin was tested for its ability to inhibit cutaneous anaphylaxsis, an allergic reaction mediated by allergen-induced degranulation of pre-sensitized mast cells. Mice were pre-sensitized to the haptenizing agent dinitrofluorobenzene (DNFB) by cutaneous application on both ears of mice followed 24 hours later by a second cutaneous application. One week later, vehicle or borrelidin was topically applied to one ear prior to DNFB challenge, and the resulting ear edema or inflammation was compared in the two ears. A topical formulation of borrelidin was used (1:2 DMSO/MeOH). Ears were harvested and neutrophil-specific myeloperoxidase (MPO) levels were determined ($p=0.000004$ for borrelidin versus vehicle treated group). As shown in Figure 15, topically applied borrelidin significantly decreased allergen-induced inflammation compared with the vehicle control-treated ear as demonstrated by a decrease in absorbance at 650nm.

Example 16. Borrelidin suppresses neutrophil recruitment into the conjunctiva in an in vivo allergic ocular conjunctivitis mouse model.

Mice were immunized on Day 0 with 100μg of ovalbumin in Alum:water (3:1) and day 6 with 50μg of ovalbumin Alum:water (3:1). On day 18, mice were challenged with 2.5μl of 100μg/ml ovalbumin after a 30 min pre-treatment in the presence(2μl of 1ng/μl or 10ng/ml of borrelidin in 2% HPMC) or absence (2μl of 2% HPMC) of borrelidin. An eye-drop formulation of borrelidin was prepared in a 2% solution of tetrahydrozoline hydrochloride The left eye receieved borrelidin treatment, the right, vehicle. 20 minutes after the challenge mice were scored on the level of swelling. 24 hours after the challenge, conjunctivas were excised. The levels of myleoid peroxidase (MPO) and total protein were deterimined on homogenized conjunctiva lysates. Figure 16 compares normalized levels of MPO of the treated eye are to the untreated eye (Mice 1-8 are

Black/6; Mice 9-10 are Balb/c; Mice 5 and 6 recieved the higher dose of borrelidin (20ng/eye); and the remaining mice recieved 2ng/eye).

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of skill in the art in light of the teachings of this invention that changes and modification may be made thereto without departing from the spirit or scope of the appended claims. All patents, patent applications and publications referred to herein are hereby incorporated by reference.

What is claimed is:

1. Use of an aminoacyl tRNA synthetase (aa-RS) inhibitor as an inhibitor of immune cells, providing that when the immune cells to be inhibited are solely T-cells and the inhibitor comprises adenine joined to a sugar molecule as a substituent, the inhibition comprises inhibition of activation of the T-cells.

2. Use of an aminoacyl tRNA synthetase (aa-RS) inhibitor for preparation of a medicament for use as an inhibitor providing that when the immune cells to be inhibited are solely T-cells and the inhibitor comprises adenine joined to a sugar molecule as a substituent, the inhibition comprises inhibition of activation of the T-cells.

3. A pharmaceutical composition for use as an inhibitor of immune cells, wherein the composition comprises a pharmaceutically acceptable carrier or excipient and an aminoacyl tRNA synthetase (aa-RS) inhibitor providing that when the immune cells to be inhibited are solely T-cells and the inhibitor comprises adenine joined to a sugar molecule as a substituent, the inhibition comprises inhibition of activation of the T-cells.

4. A pharmaceutical formulation for use as an inhibitor of immune cells, wherein the formulation is in oral, topical, parenteral or inhalation form and comprises an aminoacyl tRNA synthetase (aa-RS) inhibitor providing that when the immune cells to be inhibited are solely T-cells and the inhibitor comprises adenine joined to a sugar molecule as a substituent, the inhibition comprises inhibition of activation of the T-cells.

5. The use or composition of any one of claims 1 to 4, wherein the inhibitor is a threonyl tRNA synthetase inhibitor.

6. The use or composition of claim 5, wherein the inhibitor does not comprise adenine joined to a sugar molecule as a substituent.

7. The use or composition of claim 5 or 6, wherein the threonyl tRNA synthetase inhibitor is borrelidin, a borrelidin compound or a pharmaceutically acceptable salt thereof.

8. The use or composition of claim 5 or 6, wherein the threonyl tRNA synthetase inhibitor is borrelidin or a pharmaceutically acceptable salt thereof.

9. The use or composition of any one of claims 1 to 8 for treatment of inflammation.

10. The use or composition of claim 9, wherein the inflammation is associated with an immunological disease.

11. The use or composition of claims 9 or 10, wherein the inflammation is associated with an adaptive immune response or an innate immune response.

12. The use or composition of claim 9, wherein the inflammation is associated with an autoimmune disease.

13. The use or composition of any one of claims 9 to 12, wherein the inflammation is associated with activation of one or more of T-cells, B-cells, macrophages, mast cells, neutrophils and monocytes.

14. The use or composition of any one of claims 8 to 12, wherein the inflammation is associated with proliferation of one or more of B-cells, macrophages, neutrophils and monocytes.

15. The use or composition of any one of claims 9 to 14, wherein the inflammation is not associated with rheumatoid arthritis or psoriasis.

16. The use or composition of any one of claims 9 to 15, wherein the inflammation is not associated with microbial infection.

17. The use or composition of any one of claims 9 to 15, wherein the inflammation is not associated with infection at a site of inflammation.

18. The use or composition of any one of claims 9 to 17, wherein the inflammation is not treatable solely by use of antimicrobial therapy.

19. The use or composition of any one of claims 1 to 8, wherein the immune cell inhibition comprises prevention of activation of T-cells.

20. The use or composition of claim 19 for treatment of transplant rejection.

21. A method for treatment or prevention of an inflammatory condition or suppression of immune cells in a subject in need thereof, wherein the method comprises administering to the subject an effective amount of an amino acid tRNA synthetase (aa-RS) inhibitor, providing that when immune cells to be inhibited are solely T-cells and the inhibitor comprises adenine joined to a sugar molecule as a substituent, the inhibition comprises inhibition of activation of the T-cells.

22. The method of claim 21 for treatment or prevention of inflammation in the patient.

23. The method of claim 21 or 22, wherein said patient does not require the administration solely for treatment of psoriasis or rheumatoid arthritis.

24. The method of claim 21, wherein the treatment or prevention is for transplant rejection.

25. The method of claim 24, wherein the inhibitor is administered prior to transplantation.

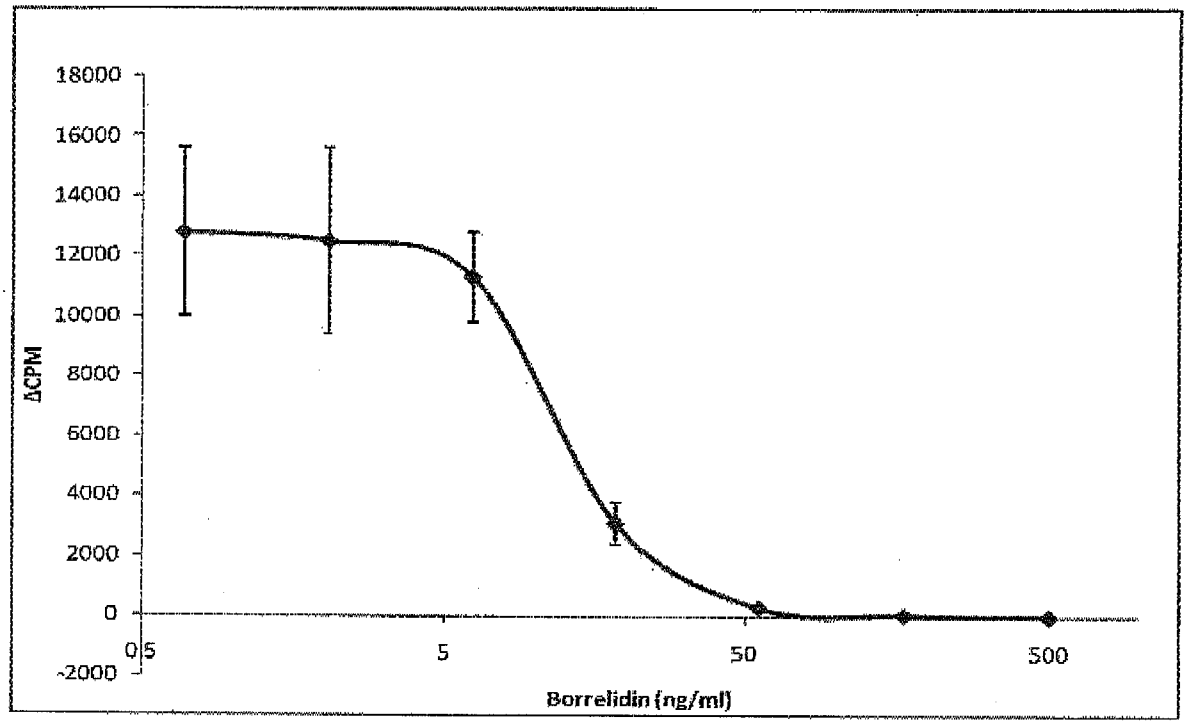

18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
ΔCPM
0.5
5
50
500
Borrelidin (ng/ml)

Figure 1

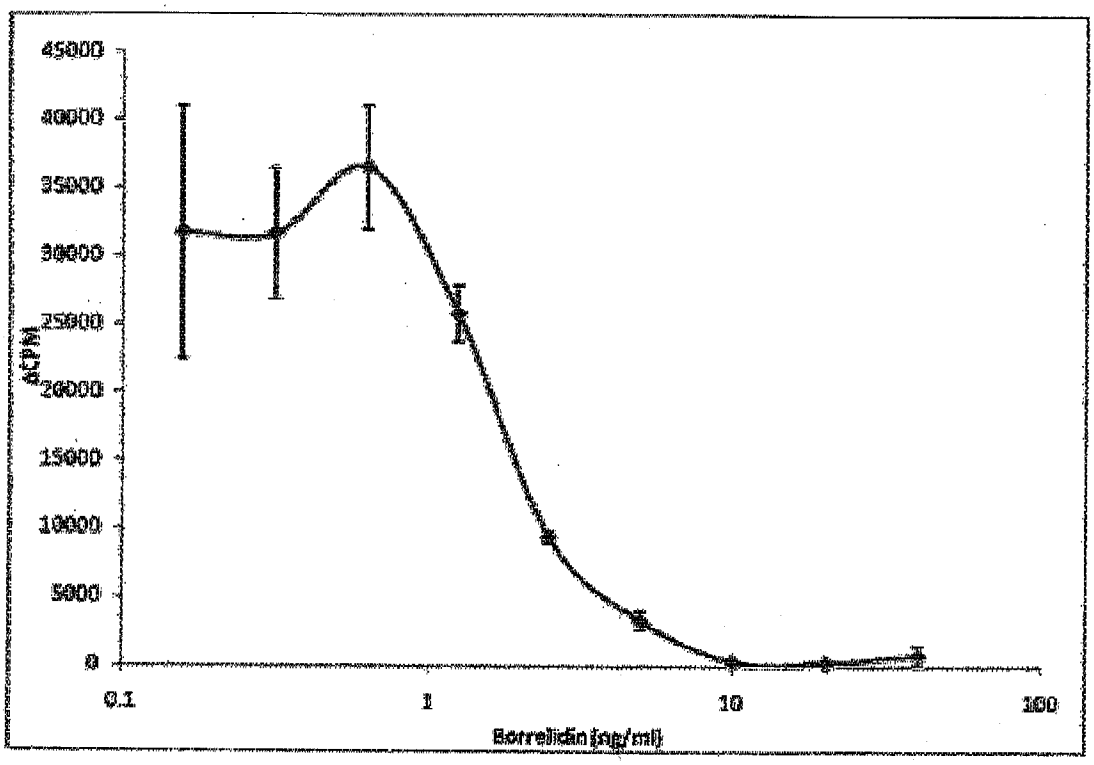

45000
40000
35000
30000
25000
20000
15000
10000
5000
0
ΔCPM
0.1
1
10
100
Borrelidin (ng/ml)

Figure 2

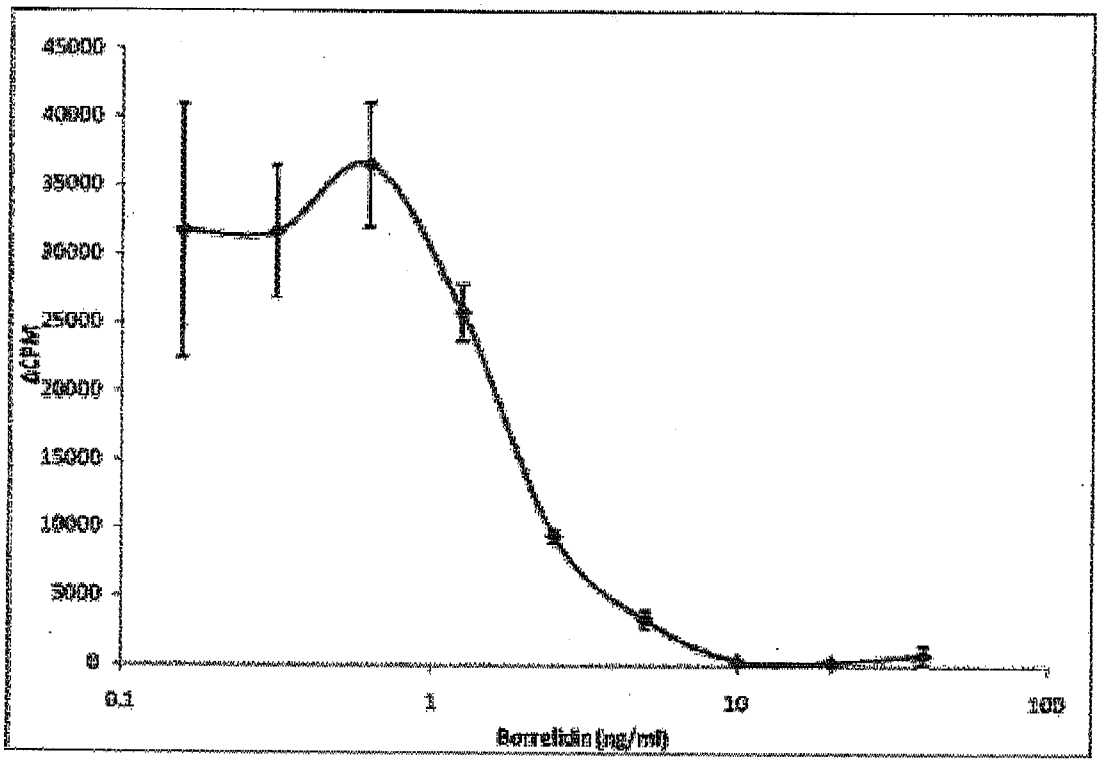

45000
40000
35000
30000
25000
20000
15000
10000
5000
0
ΔCPM
0.1
1
10
100
Borrelidin (ng/ml)

Figure 3

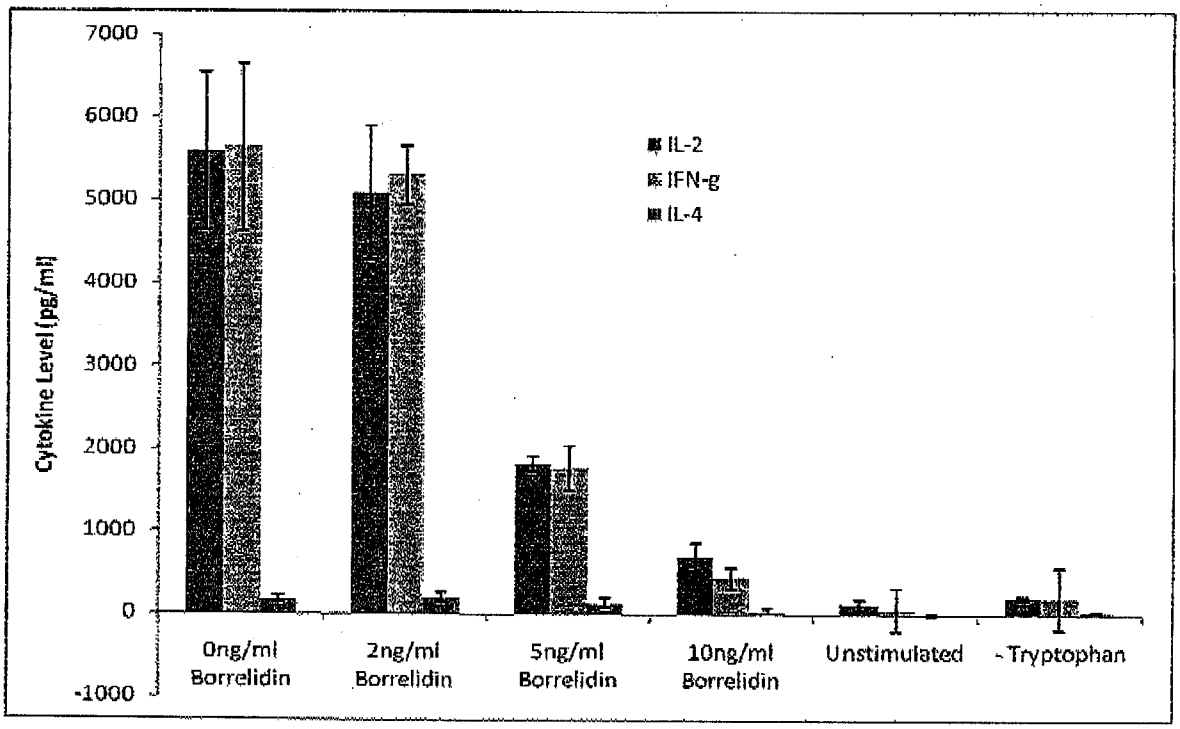

7000
6000
5000
4000
3000
2000
1000
0
-1000
Cytokine Level (pg/ml)
IL-2
IFN-g
IL-4
0ng/ml Borrelidin
2ng/ml Borrelidin
5ng/ml Borrelidin
10ng/ml Borrelidin
Unstimulated
-Tryptophan

Figure 4

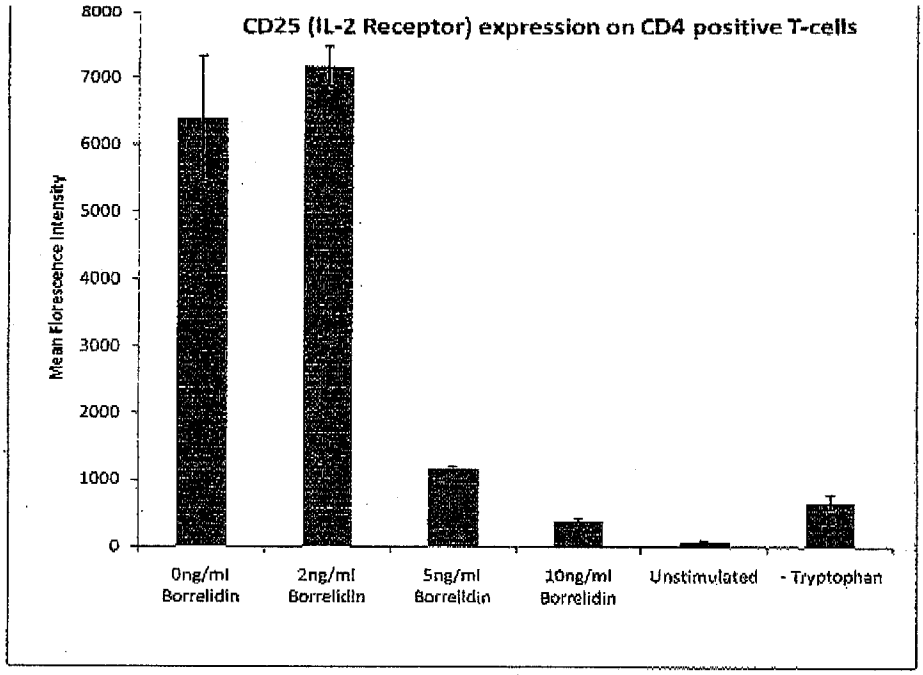


Figure 5a

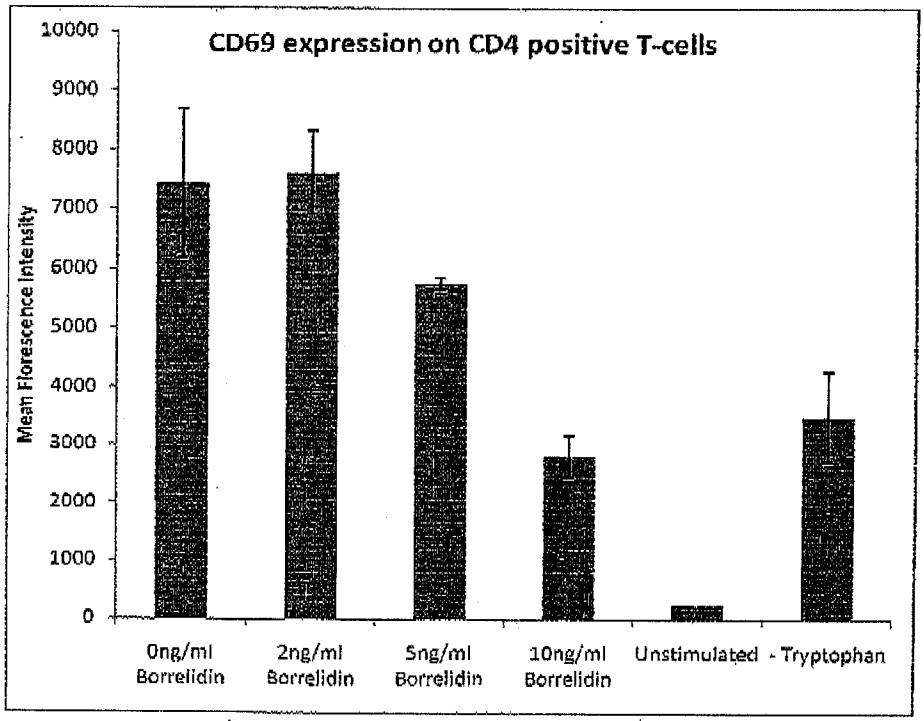


Figure 5b

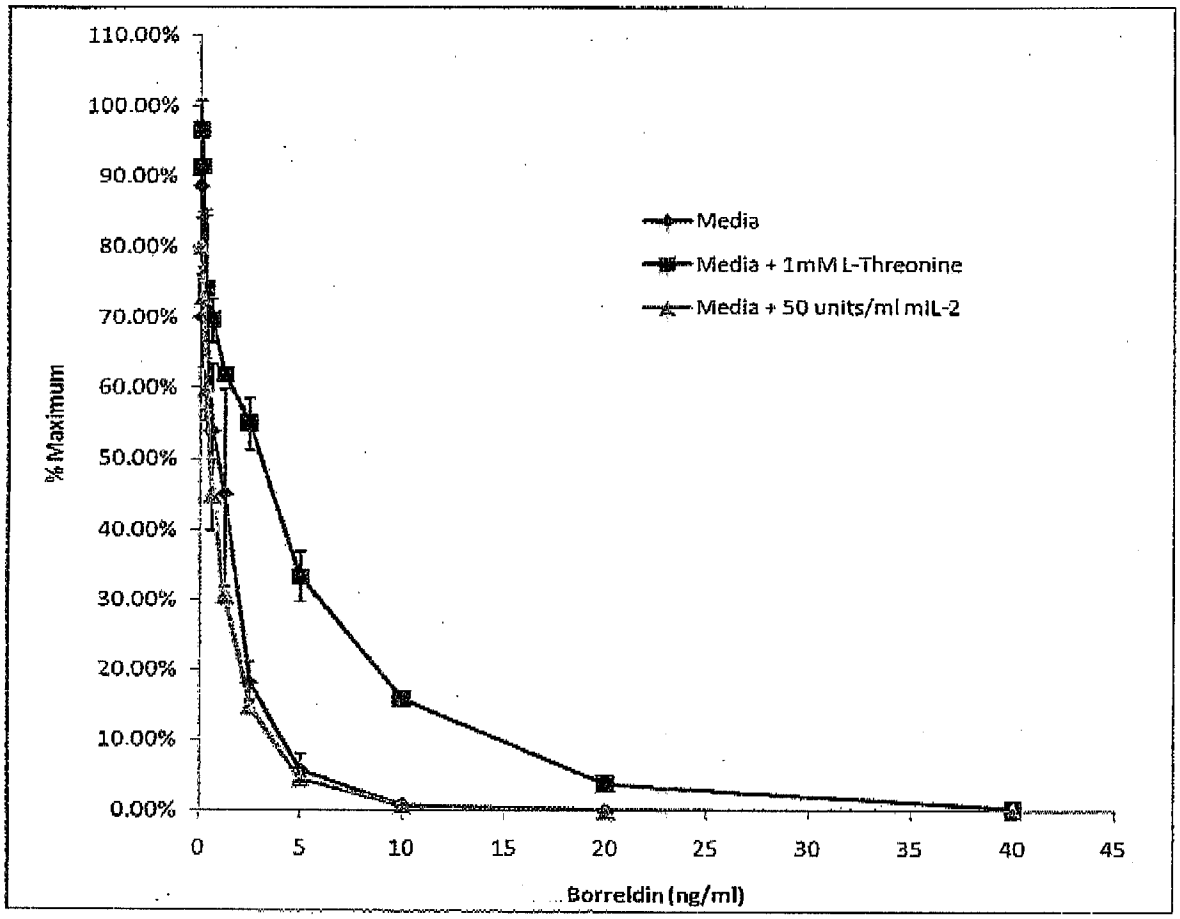

110.00%
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
% Maximum
0
5
10
15
20
25
30
35
40
45
Borreldin (ng/ml)
Media
Media + 1mM L-Threonine
Media + 50 units/ml mIL-2

Figure 6

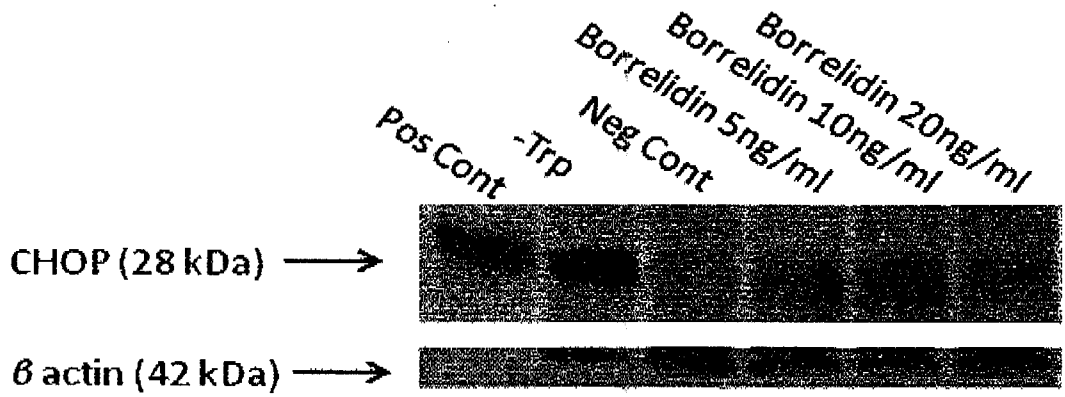

Pos Cont
-Trp
Neg Cont
Borrelidin 5ng/ml
Borrelidin 10ng/ml
Borrelidin 20ng/ml
CHOP (28 kDa)
β actin (42 kDa)

Figure 7

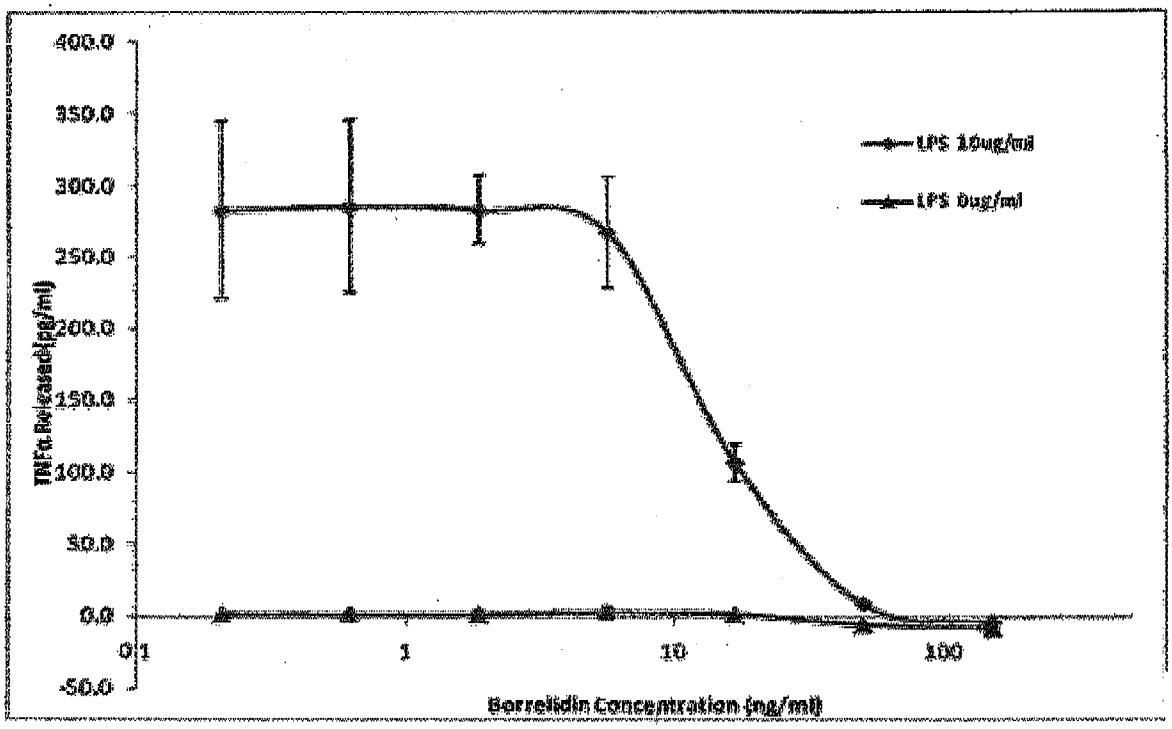

400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
-50.0
0.1
1
10
100
TNFα Released (pg/ml)
Borrelidin Concentration (ng/ml)
LPS 10ug/ml
LPS 0ug/ml

Figure 8

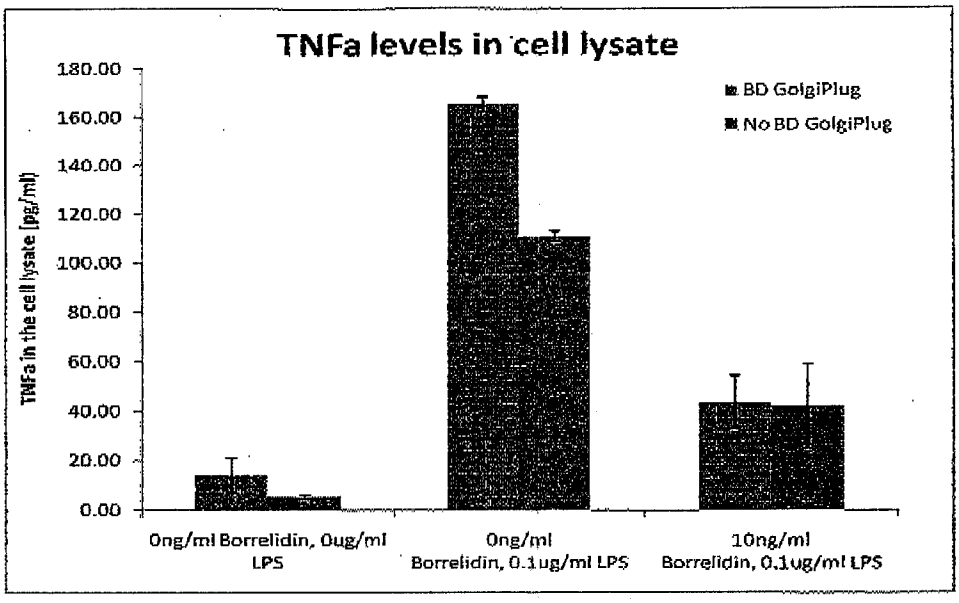


Figure 9a

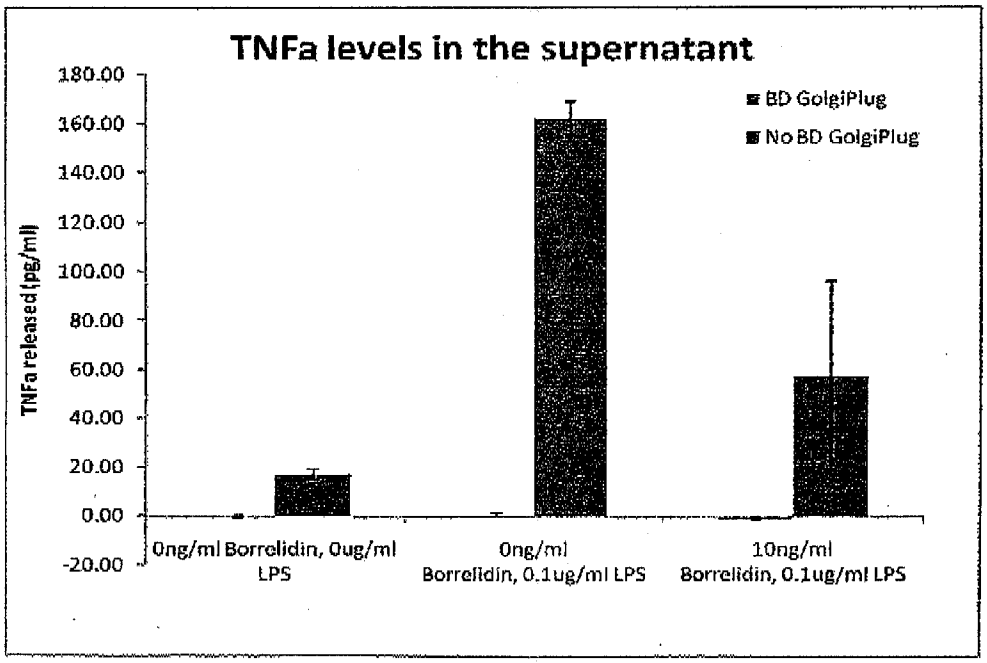


Figure 9b

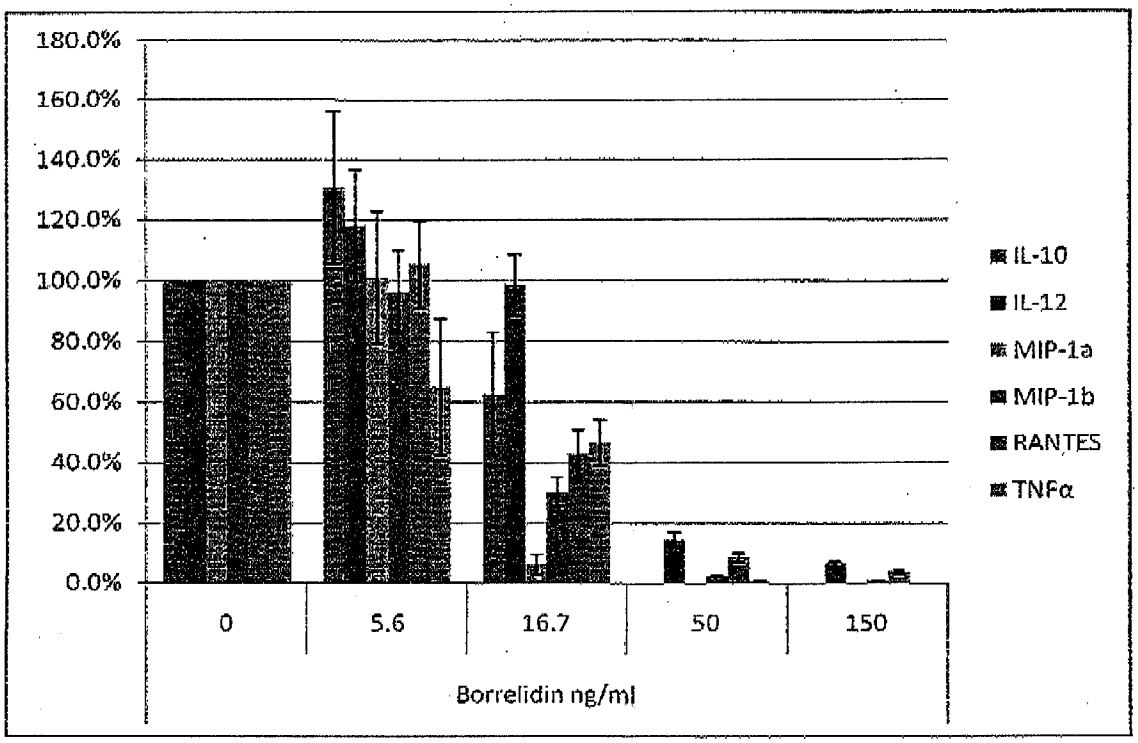

180.0%
160.0%
140.0%
120.0%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
0
5.6
16.7
50
150
Borrelidin ng/ml
IL-10
IL-12
MIP-1a
MIP-1b
RANTES
TNFα

Figure 10

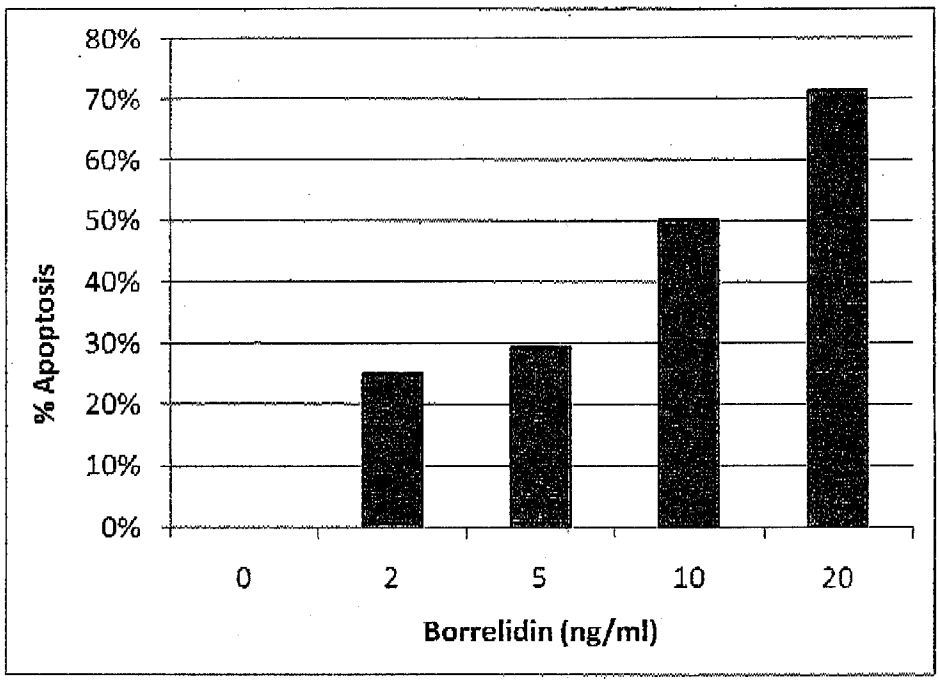


Figure 11

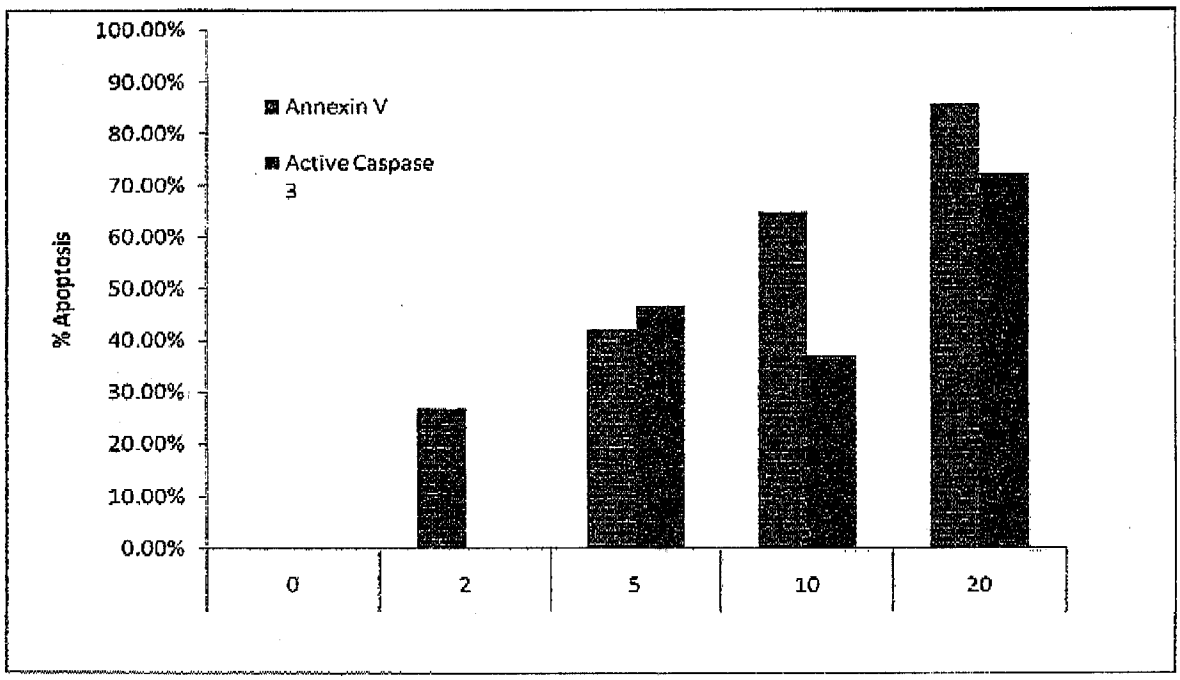

100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
% Apoptosis
Annexin V
Active Caspase 3
0
2
5
10
20

Figure 12

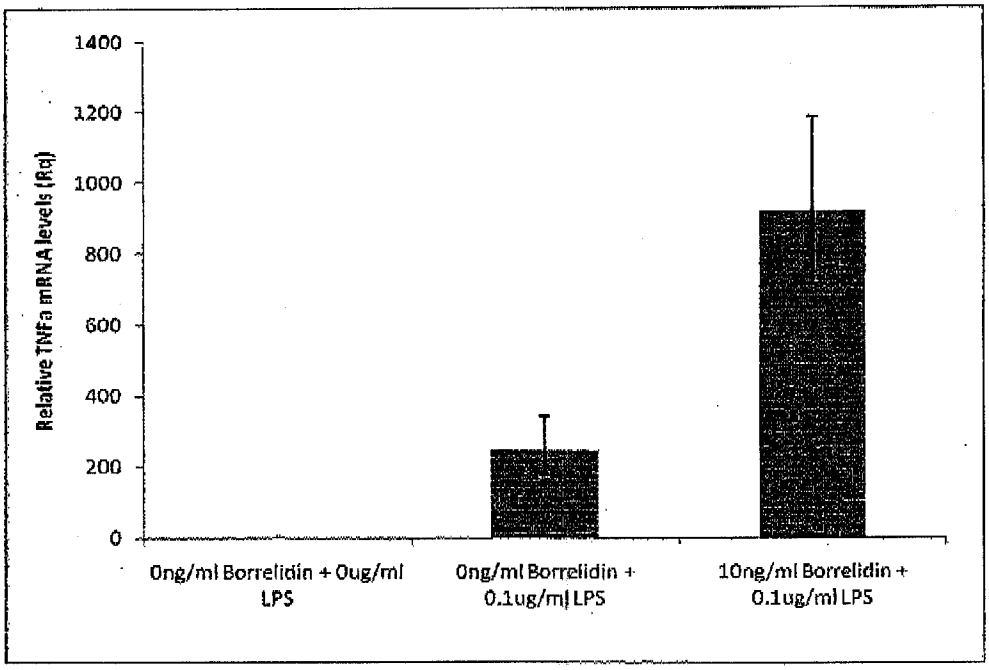


Figure 13a

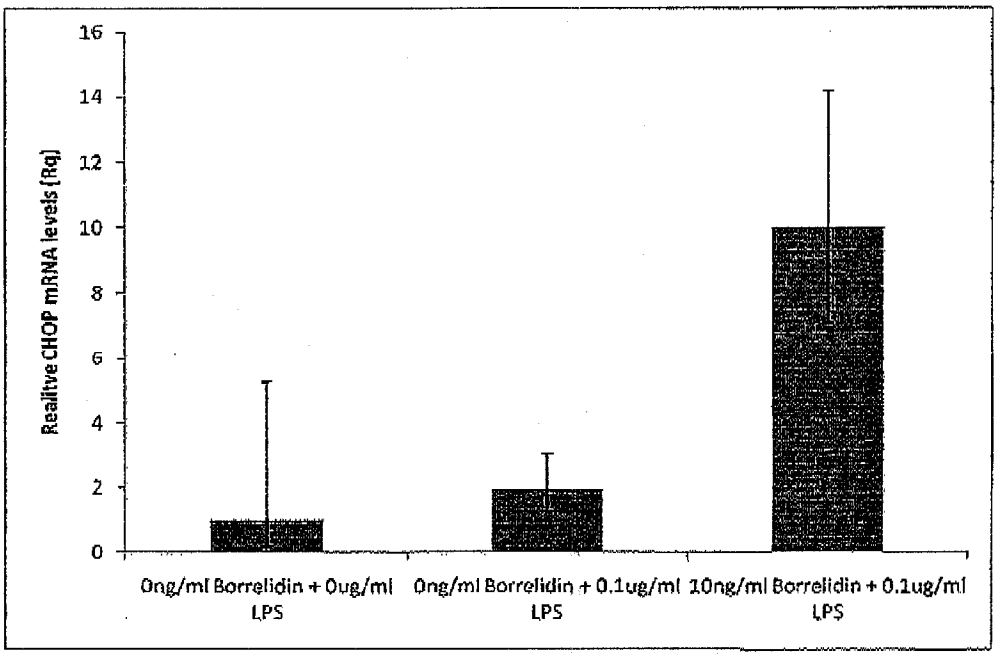


Figure 13b

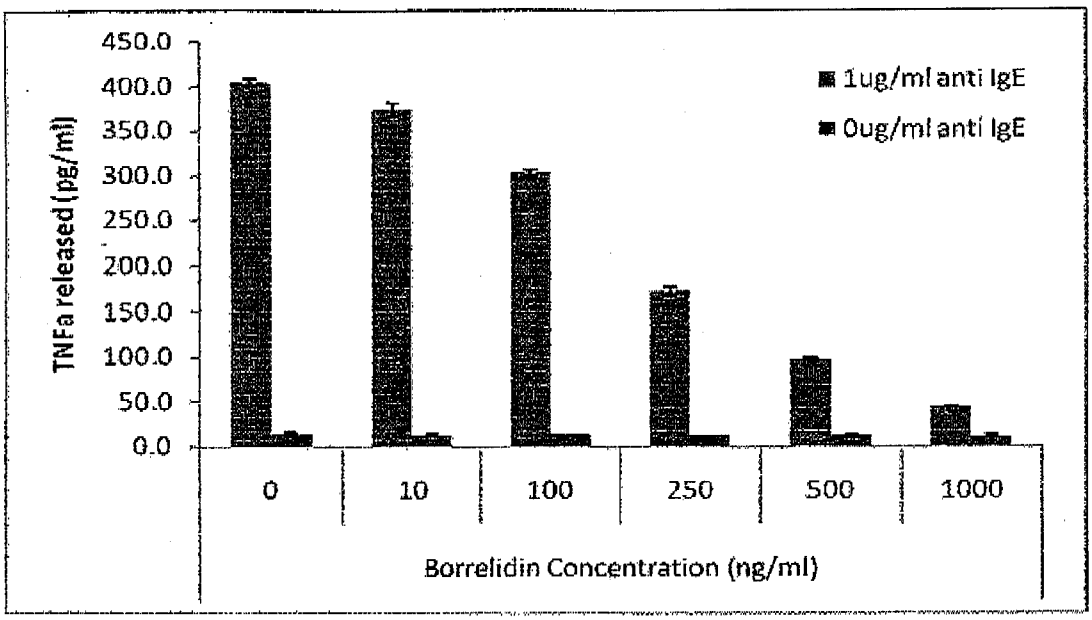

450.0
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
TNFa released (pg/ml)
1ug/ml anti IgE
0ug/ml anti IgE
0
10
100
250
500
1000
Borrelidin Concentration (ng/ml)

Figure 14

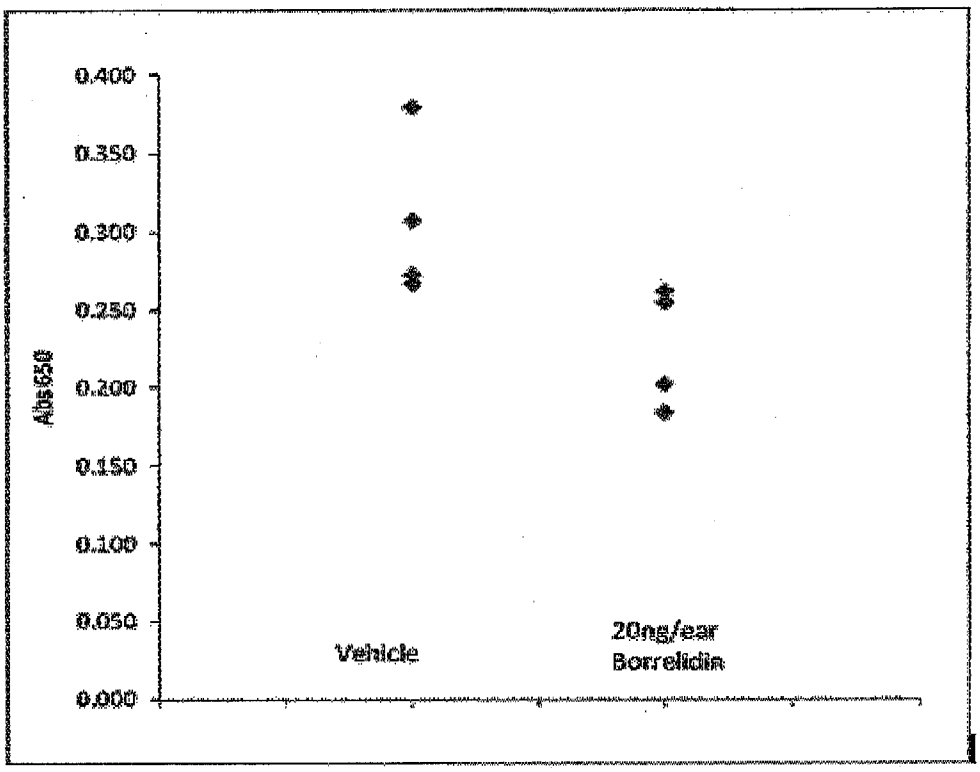

0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
Abs650
Vehicle
20ng/ear
Borrelidin

Figure 15

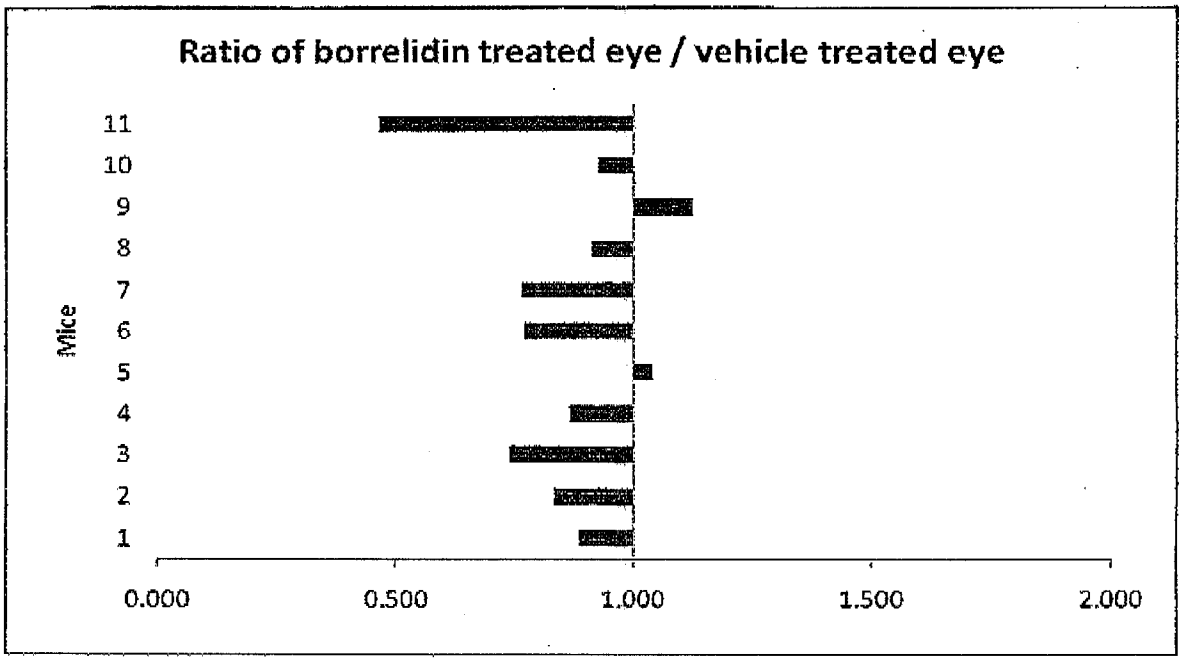

Ratio of borrelidin treated eye / vehicle treated eye
Mice
11
10
9
8
7
6
5
4
3
2
1
0.000
0.500
1.000
1.500
2.000

Figure 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/001582

A. CLASSIFICATION OF SUBJECT MATTER
IPC (2010.01): ***A61K 31/365*** , ***A61K 31/7076*** , ***A61K 45/00*** , ***A61P 29/00*** ,***A61P 37/06***

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (2010.01): ***A61K 31/365*** , *A61K 31/335* , ***A61K 31/7076*** , *A61K 31/7064* , ***A61K 45/00*** , ***A61P 29/00*** , ***A61P 37/06*** , *A61K 37/02*

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, Questel/Orbit QWeb (FAMPAT), PubMed (MEDLINE)
Keywords: borrelidin, adenine, autoimmune, immunosuppressant, inflammatory, transplant

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, Y	FRIEST, *et al.*, BIOLOGICAL CHEMISTRY HOPPE-SEYLER, Vol. 376, April 1995 (04-1995), pages 213-224. See abstract; "Borrelidin, a Specific Inhibitor of Threonyl-tRNA Synthetase" on pages 218-219; "Threonyl-tRNA and Autoimmune diseases" on pages 219-220; and "Concluding Remarks" on page 220.	X: 1-8 Y: 9-25
	--	
X	WO 96/02555 A1 (KRIEG) 1 February 1996 (01-02-1996) See page 8, lines 26-34; and page 18, lines 16-25.	3-8
	--	
X	WO 03/004511 A2 (RENZI, *et al.*) 16 January 2003 (16-01-2003) See page 11, Table 1; and examples 1 and 2.	3-8
	--	
Y	LEVINE, *et al.*, CURRENT OPINION IN RHEUMATOLOGY, Vol. 15, November 2003 (11-2003), pages 708-713. See abstract; "Aminoacyl tRNA synthetases and inflammation" on pages 710-711.	9-25
	--	
X	WO 2007/135078 A2 (WILKINSON, *et al.*) 29 November 2007 (29-11-2007) See pages 1-5.	1-8

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* 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 application or patent 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 when the document is taken alone

"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

Date of the actual completion of the international search	Date of mailing of the international search report
23 December 2009 (23-12-2009)	12 January 2010 (12-01-2010)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Philip O. Brown, Ph.D (819) 994-1622

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/001582

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 1, 5-20 (in part), and 21-25
because they relate to subject matter not required to be searched by this Authority, namely :

Said claims are directed to methods for treatment of the human or animal body by surgery or therapy which, under Article 34(4)(a)(i) and Rule 67.1(iv), the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effect or purpose/use of the products appearing in said claims.

2. [X] Claim Nos. : 1-7, 10-19, 21, and 23
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

The above claims do not clearly and concisely define the subject matter for which protection is sought, and thus fail to comply with Article 6 of the PCT. By the same token, the subject matter encompassed by said claims has not been clearly and completely disclosed in the description, such that the application does not comply with Article 5 of the PCT. See extra sheet for further details.

3. [ ] Claim Nos. :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VAN VENROOIJ, *et al.*, CURRENT OPINION IN IMMUNOLOGY, Vol. 7, December 1995 (12-1995), pages 819-824. See abstract; and "Aminoacyl-tRNA synthetases" on page 821. --	1-25
A	PARK, *et al.*, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, Vol. 105, No. 32, 12 August 2008 (12-08-2008), pages 11043-11049. See abstract; and "AAARs in Autoimmune Diseases" on page 11046. ----	1-25

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO9602555A1	01-02-1996	AT328890T	15-06-2006
		AT332966T	15-08-2006
		AT420171T	15-01-2009
		AU713040B2	18-11-1999
		AU775185B2	22-07-2004
		AU1912795A	16-02-1996
		AU5242498A	22-05-1998
		AU9724901A	07-02-2002
		AU2004218696A1	04-11-2004
		AU2004218696B2	06-11-2008
		CA2194761A1	01-02-1996
		CA2194761C	19-12-2006
		CA2270345A1	07-05-1998
		CA2560114A1	01-02-1996
		CN1235609A	17-11-1999
		CN100338086C	19-09-2007
		CN101265285A	17-09-2008
		DE10055372A1	16-08-2001
		DE69535036D1	20-07-2006
		DE69535036T2	18-01-2007
		DE69535905D1	26-02-2009
		DE69736331D1	24-08-2006
		DE69736331T2	09-08-2007
		DK0772619T3	09-10-2006
		DK0948510T3	30-10-2006
		EP0772619A1	14-05-1997
		EP0772619B1	07-06-2006
		EP0948510A1	13-10-1999
		EP0948510A4	02-08-2000
		EP0948510B1	12-07-2006
		EP1167377A2	02-01-2002
		EP1167377A3	08-09-2004
		EP1167377B1	07-01-2009
		EP1167378A2	02-01-2002
		EP1167378A3	17-08-2005
		EP1167379A2	02-01-2002
		EP1167379A3	08-09-2004
		EP1714969A2	25-10-2006
		EP1746159A2	24-01-2007
		EP1746159A3	25-11-2009
		ES2267100T3	01-03-2007
		ES2268736T3	16-03-2007
		ES2320315T3	21-05-2009
		JP3468773B2	17-11-2003
		JP4126252B2	30-07-2008
		JP10506265T	23-06-1998
		JP2001503267T	13-03-2001
		JP2003144184A	20-05-2003
		JP2003286174A	07-10-2003
		JP2004024261A	29-01-2004
		JP2004041224A	12-02-2004
		JP2004043466A	12-02-2004
		JP2004215670A	05-08-2004
		JP2008169216A	24-07-2008
		JP2008289495A	04-12-2008
		JP2009148296A	09-07-2009
		KR20000052994A	25-08-2000
		NZ335397A	24-11-2000
		PT772619E	31-10-2006
		PT948510E	29-12-2006
		TW480456B	21-03-2002
		US6194388B1	27-02-2001
		US6207646B1	27-03-2001

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO9602555A1 (continued)	01-02-1996	US6239116B1	29-05-2001
		US6429199B1	06-08-2002
		US6481860B1	19-11-2002
		US6653292B1	25-11-2003
		US7223741B2	29-05-2007
		US7402572B2	22-07-2008
		US7517861B2	14-04-2009
		US7524828B2	28-04-2009
		US2003026782A1	06-02-2003
		US2003050261A1	13-03-2003
		US2003050263A1	13-03-2003
		US2003053229A1	20-03-2003
		US2003100527A1	29-05-2003
		US2003191079A1	09-10-2003
		US2004087534A1	06-05-2004
		US2004087538A1	06-05-2004
		US2004106568A1	03-06-2004
		US2004132685A1	08-07-2004
		US2004142469A1	22-07-2004
		US2004143112A1	22-07-2004
		US2004147468A1	29-07-2004
		US2004152656A1	05-08-2004
		US2004152657A1	05-08-2004
		US2004162258A1	19-08-2004
		US2004162262A1	19-08-2004
		US2004167089A1	26-08-2004
		US2004171150A1	02-09-2004
		US2004181045A1	16-09-2004
		US2004198688A1	07-10-2004
		US2004229835A1	18-11-2004
		US2005004061A1	06-01-2005
		US2005004062A1	06-01-2005
		US2005009774A1	13-01-2005
		US2005032736A1	10-02-2005
		US2005037403A1	17-02-2005
		US2005037985A1	17-02-2005
		US2005049215A1	03-03-2005
		US2005049216A1	03-03-2005
		US2005054602A1	10-03-2005
		US2005059625A1	17-03-2005
		US2005070491A1	31-03-2005
		US2005101554A1	12-05-2005
		US2005101557A1	12-05-2005
		US2005123523A1	09-06-2005
		US2005148537A1	07-07-2005
		US2005171047A1	04-08-2005
		US2005182017A1	18-08-2005
		US2005215500A1	29-09-2005
		US2005233995A1	20-10-2005
		US2005233999A1	20-10-2005
		US2005239732A1	27-10-2005
		US2005239736A1	27-10-2005
		US2005244379A1	03-11-2005
		US2005244380A1	03-11-2005
		US2005245477A1	03-11-2005
		US2005267057A1	01-12-2005
		US2005267064A1	01-12-2005
		US2005277604A1	15-12-2005
		US2005277609A1	15-12-2005
		US2006003955A1	05-01-2006
		US2006058251A1	16-03-2006
		US2006089326A1	27-04-2006
		US2006094683A1	04-05-2006
		US2007009482A9	11-01-2007
		US2007010470A9	11-01-2007
		US2007065467A1	22-03-2007

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO9602555A1 (continued)	01-02-1996	US2007066553A1	22-03-2007
		US2007078104A1	05-04-2007
		US2007202128A1	30-08-2007
		US2008026011A1	31-01-2008
		US2008031936A1	07-02-2008
		US2009202575A1	13-08-2009
		WO9818810A1	07-05-1998
WO03004511A2	16-01-2003	AT386548T	15-03-2008
		AU2002319033B2	10-01-2008
		CA2451738A1	16-01-2003
		DE60225141D1	03-04-2008
		DE60225141T2	05-03-2009
		DK1406667T3	16-06-2008
		EP1406667A2	14-04-2004
		EP1406667B1	20-02-2008
		ES2301659T3	01-07-2008
		JP2004532900T	28-10-2004
		MXPA04000162A	22-05-2006
		NZ530360A	27-01-2006
		PT1406667E	23-05-2008
		US2005032723A1	10-02-2005
		WO03004511A3	10-07-2003
WO2007135078A2	29-11-2007	AU2007253422A1	29-11-2007
		CA2652399A1	29-11-2007
		CN101442999A	27-05-2009
		EP2023915A2	18-02-2009
		GB0609981D0	28-06-2006
		JP2009537487T	29-10-2009
		KR20090010064A	28-01-2009
		MX2008014600A	28-11-2008
		US2009312316A1	17-12-2009
		WO2007135078A3	21-02-2008

Continuation of Box II:

Claims 1-5, 7, and 21 do not clearly define the matter for which protection is sought, and therefore fail to comply with Article 6 of the PCT. In each of the above claims, components of the compositions are functionally defined in terms of a desired mechanism of action (i.e., "aminoacyl tRNA synthetase inhibitor" , "threonyl tRNA synthetase inhibitor") or structural "similarity" to a known compound (i.e., "adenine joined to a sugar molecule", "borrelidin compound"). Such expressions fail to clearly and explicitly define what compounds actually fall within the scope of these claims. Furthermore, the disclosure relating to such genera is so incomplete with regards to the meaning of Article 5 such that said claims appear to lack support with regards to the meaning of Article 6. This renders a search across the entire breadth of these claims impossible. As a result, these claims have been searched on the basis of the examples supported by the disclosure, namely compositions comprising borrelidin or adenosine containing compounds.

Claims 1-4, 10-14, 19, and 21do not clearly define the matter for which protection is sought, and therefore fail to comply with Article 6 of the PCT. In the above claims, the medicinal use is functionally defined in terms of a mechanism of action (*i.e.*, "inhibitor of immune cells", inflammation "associated" with various disorders, nature of the immune response, cells activated, and provisos relating to such). Such definitions fail to clearly and concisely state the specific pathological disorders or biological effect on an organism that is to be achieved. As a result, it is unclear how the claimed compositions and methods can be practically applied to treat pathological disorders or achieve organism-wide biological effects encompassed by these functional definitions. As a result, these claims have been searched on the basis of the applications appearing in the disclosure, namely treatment of inflammation *per se*, treatment of autoimmune disorders, and treatment of transplant rejection.

Claims 6, 15-18, and 23 do not clearly define the matter for which protection is sought, and therefore fail to comply with Article 6 of the PCT. Said claims make use of negative limitations to exclude various disorders that cause inflammation, with no positive recitations of subject matter encompassed by these claims. Such limitations fail to clearly identify what disorders are actually included in the above claims, such that a full search of these claims is not possible. Said claims have been searched on the basis of the applications appearing in the disclosure, namely treatment of inflammation *per se*, treatment of autoimmune disorders, and treatment of transplant rejection.