There is also a need for a novel therapeutic composition that treats, prevents, abates or ameliorates autoimmune disorders, inflammatory or infectious disease, myelosuppression or hematopoietic and myeloid abnormalities. The therapeutic composition should be useful as an adjuvant with other therapeutic agents. Moreover, such a therapeutic composition should be simple and relatively inexpensive to prepare, its activity should be reproducible among preparations, its activity should remain stable over time, and its effects should be achievable with *dose regimens that are associated with minimal toxicity.*

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~disclosure satisfies the above needs by providing novel compositions comprising ribonucleic acid (RNA) and cell walls (defined as RNC herein) which are derived from bacteria or mycobacteria. The present ~~invention~~disclosure satisfies the above needs by providing novel compositions comprising bacterial ribonucleic acid (RNA) and bacterial cell walls (defined as BRNC) and methods of making and using these compositions. The present ~~invention~~disclosure also satisfies the above needs by providing novel compositions comprising mycobacterial RNA and mycobacterial cell walls (defined as MRNC) and methods of making and using these compositions. These novel compositions comprise bacterial or mycobacterial RNA in the form of oligoribonucleotides and polyribonucleotides wherein the RNA can exist as single strands (ss), double strands (ds), or single-double stranded hybrid strands (hs), isolated from bacteria or mycobacteria and bacterial or mycobacterial cell walls, wherein the RNA is formulated in a pharmaceutically acceptable vehicle or complexed (where the term complex is used to describe the chemical association of two or more molecular species) to a pharmaceutically acceptable carrier system. Although pharmaceutical carrier systems known to those of skill in the art can be used, in one embodiment the carrier system is based on bacterial cell walls or mycobacterial cell walls. The compositions of the present ~~invention~~disclosure may also contain intact bacterial or mycobacterial cells, the content of which is selected for the desired therapeutic intent.

In one aspect, there is provided a composition comprising:

mycobacterial RNA isolated from mycobacteria cells: and,

mycobacterial cell walls isolated from mycobacterial cells, wherein the composition does not contain phenol or pronase and the RNA is in the form of oligoribonucleotides and polyribonucleotides, wherein the length of the oligoribonucleotides and polyribonucleotides is 2 to 150 bases.

The compositions of the present ~~invention~~disclosure possess anti-cancer activity. The compositions of the present ~~invention~~disclosure also contain immune system stimulating activity. The compositions of the present ~~invention~~disclosure also act as adjuvants to other therapeutic modalities.

Three different names are used in the present application to describe these novel compositions. First, the term bacterial RNA composition (BRNC) is used generally to describe

the compositions made from different species of bacteria. BRNC may be prepared from different Gram-negative or Gram-positive bacteria with the methods disclosed herein. Second, the term mycobacterial RNA composition (MRNC) is used to describe the compositions made from different mycobacteria. In different embodiments, MRNC may be prepared from any mycobacterium. Third, in other embodiments, specific mycobacterial species, mycobacterial complexes or mycobacterial strains are used to prepare MRNC. Examples of but not limited to these are *Mycobacterium avium* (including sub-species *paratuberculosis*, commonly termed MAP), *Mycobacterium bovis* BCG, *Mycobacterium phlei, Mycobacterium smegmatis*, or *Mycobacterium vaccae* with the methods disclosed herein. It will be recognized by one of skill in the art that the methods of manufacturing the compositions described herein are applicable to the manufacture of MRNC from mycobacteria other than those described in the detailed description and examples.

In another aspect, there is provided a method of making the composition as described herein, comprising the sequential steps of:

A) disrupting a mycobacterial cell biomass to generate disrupted mycobacterial cells, intact mycobacterial cells, disrupted mycobacterial cell walls and RNA;

B) optionally separating intact mycobacterial cells from the disrupted mycobacterial cell walls and RNA; and,

C) separating the soluble, cytosolic contents of a portion of the disrupted mycobacterial cells from the disrupted mycobacterial cell walls and RNA to obtain a mycobacterial cell wall, ribonucleic acid composition; and

D) heating at a temperature sufficient to generate oligoribonucleotides and polyribonucleotides of 2 to 150 bases in length, wherein the intact mycobacterial cells are inactivated.

The term *Mycobacterial* RNA composition (MpRNC) is used to describe the compositions comprising RNA and cell walls made from *Mycobacterium phlei.* The terms *Mycobacterium bovis* strain BCG RNA composition (MbRNC), *Mycobacterium smegmatis* RNA composition (MsRNC), *Mycobacterium avium* subspecies *paratuberculosis* (MAP) RNA composition (MapRNC) and *Mycobacterium vaccae* RNA composition (MvRNC) are used to describe the compositions comprising RNA and cell walls made from *Mycobacterium bovis* BCG, *Mycobacterium smegmatis*, *Mycobacterium avium* subspecies *paratuberculosis* and

Mycobacterium vaccae, respectively. In each composition, MRNC, MpRNC, MbRNC, MsRNC, MapRNC and MvRNC, RNA is isolated from mycobacteria using a procedure that results in an efficient production of oligoribonucleotides and polyribonucleotides.

In another embodiment, each MRNC, MpRNC, MbRNC, MsRNC, MapRNC or MvRNC, may also contain different amounts of intact mycobacterial cells. Intact cells may be present in MRNC, MpRNC, MbRNC, MsRNC, MapRNC or MvRNC in amounts of from approximately 0-99%, 0.05-95%, 0.07-50%, 0.1-20% or 0.19-19% by weight, or in an amount within any of these ranges, or less than 0.75% or less than 0.2% by weight.

In another embodiment, these novel compositions possess an RNA content (which can range from approximately 30% to 100% of the total nucleic acid content) in the form of oligoribonucleotides and polyribonucleotides that are 2 to 4000 bases in length. In another embodiment, these novel compositions possess an oligoribonucleotide and polyribonucleotide

Microbiol., 2005; 7:53-61), these new compositions have the ability to activate both the nucleotide-binding oligomerization domain 2 (NOD2) and Toll-like receptor 2 (TLR2) receptors, thus demonstrating an unexpected bifunctional agonist activity for immune system receptors that is useful for stimulation of the immune system, stimulating hematopoietic and myeloid stem cell proliferation and in treating cancer in both prophylactic or therapeutic treatment regimens. In different embodiments, these compositions are effective in inducing a response in responsive cells of an animal's immune system, in inducing cell cycle arrest or apoptosis and inhibiting cellular proliferation. These compositions induce responsive cells of the immune system to produce cytokines, chemokines and hematopoietic and myeloid stem cell growth factors.

MRNC, MpRNC, MbRNC, MsRNC, MapRNC and MvRNC are relatively inexpensive to prepare and their activity is reproducible among preparations. MRNC, MpRNC, MbRNC, MsRNC, MapRNC and MvRNC remain stable and effective over time and at dose regimens that are associated with minimal toxicity.

In one embodiment of the ~~invention~~disclosure, MRNC, MpRNC, MbRNC, MsRNC, MapRNC and MvRNC, are prepared from mycobacteria or *Mycobacterium phlei, Mycobacterium bovis* BCG, *Mycobacterium smegmatis*, *Mycobacterium avium* subspecies *paratuberculosis or Mycobacterium vaccae* respectively, as follows: the mycobacteria are grown and harvested. The mycobacteria are disrupted to remove as required intact mycobacteria via the utilization of high-pressure homogenization followed by a centrifugation process to eliminate as required any residual intact mycobacterial cells. Importantly, use is made of RNase-free and or non-specific nuclease-free reagents to optimize recovery of the RNC.

Low relative centrifugal force is used to remove intact mycobacteria as required. Oligoribonucleotides and polyribonucleotides can then be isolated using conventional extraction techniques, for example guanidinium thiocyanate-phenol-chloroform extraction, or a high relative centrifugal force is applied to the supernatant after removal of intact mycobacteria to isolate oligoribonucleotides and polyribonucleotides that remain formulated with mycobacterial cell walls after cellular disruption. Importantly, reagents that do not contain nuclease contamination (non-specific endo- and exo-nucleases or ribonucleases) are used to eliminate or minimize RNA degradation and thereby optimize yield during the preparation steps.

MRNC, MpRNC, MbRNC, MsRNC, MapRNC and MvRNC are effective therapeutic agents in preventing, treating, lessening the impact of and eliminating a variety of diseases

including, but not limited to, malignant, autoimmune and immunodeficiency diseases, myelosuppression and hematopoietic and myeloid abnormalities. MRNC, MpRNC, MbRNC, MsRNC, MapRNC and MvRNC are particularly useful for treating diseases and processes mediated by undesired and uncontrolled cell proliferation, such as cancer. These compositions are also effective as adjuvants to enhance the effectiveness of other anti-cancer agents. Such anti-cancer agents include, but are not limited to, drugs, immune stimulants, antigens, antibodies, vaccines, radiation, chemotherapeutic agents, genetic, biologically engineered and chemically synthesized agents, and agents that target cell death molecules for activation or inactivation, agents that inhibit proliferation of cancer cells, and that induce apoptosis in cancer cells. MRNC, MpRNC, MbRNC, MsRNC, MapRNC and MvRNC are also effective for the prevention or treatment of myelosuppression (monocytopenia and or neutropenia) associated with the treatment of cancer or cancer by itself, and for the prevention or treatment of diverse hematopoietic and myeloid abnormalities associated with medications or diseases, such as, but not limited to the acquired immunodeficiency syndrome (AIDS), myelodysplastic syndromes, autoimmune diseases, end-stage renal diseases or viral infections.

MRNC, MpRNC, MbRNC, MsRNC, MapRNC or MvRNC, in a pharmaceutically acceptable carrier, may be administered to an animal or human in a dosage effective to stimulate a response in responsive cells of the immune system, and to inhibit proliferation of and induce apoptosis in responsive cells. MRNC, MpRNC, MbRNC, MsRNC, MapRNC or MvRNC can be administered by methods including, but not limited to, suspension in aqueous formulations, in creams and gels, by emulsification in oil or other hydrophobic liquid formulations, enclosure in liposomes, and complexation with natural or artificial carriers, with tissue- or cell-specific ligands or with tissue- or cell-specific antibodies.

In one embodiment, the present ~~invention~~disclosure provides novel compositions comprising, BRNC, MRNC, MpRNC, MbRNC, MsRNC, MapRNC or MvRNC, and methods of making these compositions.

In another embodiment, the present ~~invention~~disclosure provides novel manufacturing procedures for the preparation of BRNC, MRNC, MpRNC, MbRNC, MsRNC, MapRNC or MvRNC compositions that optimize the content of carbon, nitrogen and iron sources in the cultivation media for the manufacture of mycobacterial cell mass.

In another embodiment, the present ~~invention~~disclosure provides novel synthetic media useful for growing mycobacterial cell mass.

In yet another embodiment, the present ~~invention~~disclosure provides novel manufacturing procedures for the preparation of BRNC, MRNC, MpRNC, MbRNC, MsRNC, MapRNC or MvRNC compositions that optimize the content of carbon, nitrogen and iron in the cultivation media and that eliminate the requirement for exogenous biological materials for the manufacture of mycobacterial cell mass, and that eliminate the use exogenous biological or chemical agents during the down-stream manufacturing procedure.

In still another embodiment, the present ~~invention~~disclosure provides novel therapeutic BRNC, MRNC, MpRNC, MbRNC, MsRNC, MapRNC or MvRNC, in a pharmaceutically acceptable carrier.

In a further embodiment, the present ~~invention~~disclosure provides therapeutic compositions comprising BRNC, MRNC, MpRNC, MbRNC, MsRNC, MapRNC or MvRNC, and methods of using these therapeutic compositions to induce a therapeutic response in responsive cells of an animal or a human.

In another embodiment, the present ~~invention~~disclosure provides a method to stimulate responsive cells of the immune system by administering these therapeutic compositions to an animal or a human.

In yet another embodiment, the present ~~invention~~disclosure provides a method to stimulate responsive cells of the immune system to produce bioactive molecules such as cytokines, chemokines, interleukins and/or hematopoietic and myeloid growth factors by administering these therapeutic compositions to an animal or a human.

In a further embodiment, the present ~~invention~~disclosure provides a method that is effective to treat a disease in an animal or human by administering these therapeutic compositions to an animal or a human.

In still another embodiment, the present ~~invention~~disclosure provides a method that is effective to treat cancer in an animal or human by administering these therapeutic compositions to an animal or a human.

In another aspect, there is provided use of the composition as described herein in the manufacture of a medicament for the treatment of cancer.

In another embodiment, the present ~~invention~~disclosure provides a composition and method that inhibits proliferation of responsive cells of an animal.

In another embodiment, the present ~~invention~~disclosure provides a composition and method that induces apoptosis in responsive cells of an animal.

In another aspect, there is provided a composition as described herein for use as an adjuvant to cancer therapy or immune stimulant therapy.

In yet another embodiment, the present ~~invention~~disclosure provides composition and method that is effective as an adjuvant to other anti-cancer therapies.

In still another embodiment, the present ~~invention~~disclosure provides a composition and method that is effective as an adjuvant to other immune stimulatory therapies.

In another aspect, there is provided use of the composition as described herein in the manufacture of a medicament for stimulating an immune system.

In a further embodiment, the present ~~invention~~disclosure provides a composition and method that is effective for the treatment of immunodeficiency diseases.

In yet another embodiment, the present ~~invention~~disclosure provides a composition and method that is effective for the prevention or treatment of myelosuppression (leucopenia, neutropenia, thrombocytopenia or anemia) or hematopoietic and myeloid abnormalities.

In still another embodiment, the present ~~invention~~disclosure provides a composition and method that is effective as an adjuvant to other therapies for the prevention or treatment of myelosuppression or hematopoietic and myeloid abnormalities.

In another aspect, there is provided use of the composition as described herein in the manufacture of a medicament for stimulating the synthesis of hematopoietic growth factors.

In another embodiment, the present ~~invention~~disclosure provides a method that is effective to treat an autoimmune disease in an animal or human by administering these therapeutic compositions to an animal or a human.

In yet another embodiment, the present ~~invention~~disclosure provides a method of manufacturing that does not result in the presence of chemicals or enzymes in the composition.

These and other objects, features and advantages of the present ~~invention~~disclosure will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1. Transmission electron micrographs (TEM) of *Mycobacterium phlei* (1a), MCWE (1b), MCC (1c), MpRNC Low (1d), MpRNC Intermediate (1e) and MpRNC High (1f). The bars are 1 μm for 1a, 1b and 1c, and 2 μm for 1d, 1e and 1f.

FIGURE 2. Electrophoretic analysis of nucleic acid oligonucleotide length. 2a. RNA ladder standard and MpRNC Intermediate prior to autoclaving using the RNA nano 6000 kit. 2b. MpRNC containing varying proportions of autoclaved *M. phlei* cells (MpRNC High-, MpRNC Low- or and MpRNC Intermediate-) following autoclaving using the small RNA kit (The peak at