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(54) **TUBERCULOSIS VACCINE AND METHOD OF USING SAME**

TUBERKULOSEIMPFSTOFF UND VERWENDUNGSVERFAHREN

VACCIN CONTRE LA TUBERCULOSE ET PROCÉDÉ D'UTILISATION DUDIT VACCIN

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## Description

### Field of the Invention

**[0001]** The invention relates to a vaccine against tuberculosis and more particularly to a vaccine using inactivated whole Mycobacterium tuberculosis formulated for pulmonary and mucosal delivery.

### Background of the Invention

**[0002]** Mycobacterium tuberculosis (M. tb) infects one third of the world's human population[1]. The common tuberculosis (TB) vaccine known as the BCG vaccine is given to neonates in developing countries. While this vaccine protects against meningeal and disseminated TB in children, it fails to adequately protect the establishment of latent TB or reactivation of pulmonary disease in adult life[2]. Moreover, BCG effectiveness is reported to decline over a period of 10-15 years[3]. The most common type of tuberculosis disease is pulmonary and transmission occurs via aerosol droplets expressed during coughing. Thus, despite the high prevalence of BCG vaccination, the disease burden has not decreased. There is now evidence to support that M. tb microbacterial lineages may have adapted to mutations in antigens common to both M. tb and BCG[4,5]. Moreover, recent studies suggest that BCG delivered parenterally may fail to induce T-cell immune responses in the lung mucosa, which may be critical for protection against pulmonary disease [6,7]. Given these reasons, a new vaccine is imperative to decrease the prevalence of TB throughout the World.

**[0003]** WO 00/47225 relates to a composition comprising adjuvant and mycobacteria which are inactivated either by heat or formalin for making a mucosal vaccine.

### Summary of the Invention

**[0004]** The invention provides a vaccine for preventing and/or treating tuberculosis. The invention can be utilized with a number of vaccination strategies: prophylactically-given prior to infection to prevent infection with M. tb, post-exposure to eliminate or contain latent TB and prevent reactivation. It can either be used to replace BCG and/or as a booster to BCG in patients who have already received BCG or another subunit TB immunostimulant.

**[0005]** In one aspect, the invention provides a pharmaceutical composition comprising inactivated whole *Mycobacterium tuberculosis (M. tb),* wherein the composition is formulated for intranasal, mucosal or intrapulmonary delivery to a mammalian host, and wherein the composition comprises an immunologically protective dose when delivered to the host and wherein said *M. tb* is inactivated with irradiation.

**[0006]** In some embodiments, at least 90% of the Mycobacterium spp. cells are inactivated, e.g., 95%, 98%, 99%, or 100% of the Mycobacterium spp. cells. When the subject is a human, 100% of the Mycobacterium spp. cells are preferably inactivated.

**[0007]** Preferably irradiation is with gamma irradiation.

**[0008]** The pharmaceutical composition may optionally include an adjuvant to enhance an immune response in the host.

**[0009]** The pharmaceutical composition may optionally include a pharmaceutically acceptable carrier, or be provided lyophilized.

**[0010]** In some embodiments, the pharmaceutical composition is formulated for intranasal delivery to the host.

**[0011]** In addition, the pharmaceutical composition is provided as an aerosol or spray package.

**[0012]** In one embodiment, the invention provides a pharmaceutical composition that includes a gamma-irradiated Mycobacterium spp. that is formulated for intranasal or intrapulmonary delivery to a mammalian host and which confers an immunologically protective dose when delivered to the host, e.g., a human.

**[0013]** In some embodiments, the pharmaceutical composition is delivered through a device configured for nasal or pulmonary delivery.

**[0014]** In a still further aspect, the invention provides a method for preparing a vaccine for treating Mycobacterium infection, comprising formulating an immunologically protective dose of an inactivated whole *Mycobacterium tuberculosis* for intranasal or pulmonary delivery to a mammalian host.

**[0015]** In some embodiments, the method includes testing the vaccine in a non-human animal model of tuberculosis. The animal model can be, e.g., a mouse, guinea pig, rabbit, bovine, or non-human primate.

**[0016]** Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

**[0017]** Other features and advantages of the invention will be apparent from the following detailed description and claims.

### Detailed Description of the Invention

**[0018]** A vaccine according to the invention is prepared using inactivated *Mycobacterium tuberculosis* that is then formulated for pulmonary and mucosal delivery to a subject. The inactivated mycobacterium, when delivered to the lung or mucosal/nasal mucosa of a subject is postulated to elicit a much stronger immune response than has been observed with previously described Tb vaccines.

**[0019]** Research in an influenza murine model suggests that pulmonary immune cells remain localized and only a few B cells and T cells migrate systemically.[8,9]

The research shows that key influenza-specific CD8-T cells can remain locked within a semi-isolated circuit within the chest, barely reaching the bloodstream or the peripheral lymphoid tissue but instead cycling between the respiratory mucosa and the local lymph nodes. Zammit et al suggest that one reason may be the special anatomy of the lung lymphatic drainage[8]. Cells entering the thoracic duct from the local pulmonary nodes are fed to the lung in the pulmonary arterial blood. Some may pass through to the systemic circulation, but activated cells tend to adhere to the vascular endothelium and move back into the lung, thus keeping cells at the site of infection. From here the cells again move to the local nodes where they re-encounter antigen. Indeed, it has been found in the murine TB model that antigen specific memory T-cells preferentially home back to the site of vaccination and that the location of T cells in the airway at the time of infection is of importance[10-11].

**[0020]** Applying these findings to the instant invention, then, for a TB vaccine to be successful in evoking a protective immune response in the pulmonary and respiratory mucosal system, it preferably directly stimulates the antigen-presenting cells in the respiratory epithelium. The invention accomplishes this by delivering irradiated mycobacterium directly to the pulmonary and mucosal interface.

**[0021]** One study published in 1968 reported no adverse effects when aerogenic BCG was given to 439 children.[12] In experimental animal species, aerosol or intratracheal delivery of BCG varied in efficacy from superior protection than parenteral inoculation in primates[13], cattle[14] guinea pigs[15r], and mice[16,17,18,19] to no apparent advantage over the subcutaneous route in other studies[20]. Other studies showed immune response was dependent on initial BCG inoculum dose[12,21].

**[0022]** Recently, several research groups have published data on using mucosal M tb subunit vaccines as booster when administered weeks after primary immunization in the murine model. Goonetilleke et al findings support the importance of homing properties of T cells when exposed to recombinant modified vaccinia virus Ankara, expressing Mycobacterium tuberculosis Ag 85A. Intranasal boosting induced a five fold higher T cell response in the lungs than parenteral BCG thereby providing support that T cells in the lungs are in some form compartmentalized[22]. Santosuosso et al showed that an intranasal adenoviral vector expressing Ag85A boosted primary CD4 and CD8 T-cell response in the airway lumen and enhanced protection against pulmonary M. tuberculosis challenge[23]. Other studies in mice using mycobacterial antigens (Ag 85A or Ag 85B-ESAT-6) in either recombinant bacterial / viral vectors or with proteins and adjuvants given mucosally as a booster have shown protective immunity when compared to standard parenteral BCG when challenged with live M.tb[24,25,26]. All of these studies showed statistically fewer colony forming units of mycobacteria in the lungs and spleen after the mucosal subunit vaccine boost when compared to BCG alone.

**[0023]** The adaptive immune response to live M tuberculosis infection is delayed compared to other infections and this allows the bacilli population in the lungs to markedly increase during the preimmune phase of the infection[27]. By using dead baciili in an aerosolized vaccine formulation there is no multiplying mycobacteria and the immune response would have adequate time to respond to the antigens on the cell wall of the bacteria. In addition, over thousands of years through fitness challenges M tb has found many ways to evade the innate immune response during initial antigen presentation [28,29,30,31]. Dead mycobacteria do not have the ability to produce enzymes that evoke ways to evade the human immune system and avoid successful antigen presentation.

**[0024]** One reason we believe this method of using killed whole mycobacterium has been overlooked in the past is due to studies performed by Robert Koch in the late 19$^{th}$ century[32]. Koch used a sterile filtrate from M. tuberculosis cultures as a therapeutic vaccine in subjects. This induced such a severe inflammatory immune response in some individual's with active disease, that some died. Known as the Koch phenomenon, this necrotic reaction appears to be due to overproduction of several pro-inflammatory cytokines but in particular TNF-$\alpha$[33]. This incident haunted vaccinologists for decades and we believe scientists have since overlooked the potential use of whole bacilli. Whole killed mycobacterium will be utilized in low enough quantities to avoid an overwhelming inflammatory reaction and yet still elicit a strong immunoprotective response

**[0025]** In general, any type of inactivation procedure can be used as long as the treatment leaves the population of bacteria unable to produce a productive infection at the host, while at the same time preserving antigenic structures necessarily for eliciting a productive response to the corresponding disease-causing mycobacterium. The mycobacterium preparation is typically incapacitated. By "incapacitated" in the context of an incapacitated bacterial cell produced according to the invention, is meant that the bacterial cell is in a state of irreversible bacteriostasis. While the bacterium retains its structure--and thus retains, for example, the immunogenicity, antigenicity, and/or receptor-ligand interactions associated with a wild-type bacterium--it is not capable of replicating. In some embodiments, it is incapable of replication due to the presence of an infecting phage with in the bacterial cell.

**[0026]** A preferred type of inactivation is gamma-irradiation. Other types of inactivation known in the art include, e.g., other types of radiation (including ultra-violet irradiation). In some embodiments, >70% killed for non-human use. In the embodiments for human use, 100% of the cells are killed.

**[0027]** While not wishing to be bound by theory, it is postulated that gamma-irradiated Mycobacterium are especially suitable for use in the compositions and methods of the invention. Gamma-irradiated bacteria are com-

monly used in the laboratory because they are considered safe and do not replicate. In many trials, they have nevertheless been shown to elicit an immunoprotective response, including responses elicited by antigens on the bacilli wall[34,35,36]. In addition, gamma irradiated mycobacterium undergo apoptosis and become engulfed by dendritic cells. Dendritic cells present the mycobacterium antigens to T-cells, which activate CD4 Th1 and CD8 cytotoxic cells. Gamma-irradiated M tb can also induce nitric oxide release[34] and can elicit similar Th2 responses to live M tb[35]. In 1963, Nishihara et al intradermally injected gamma-irradiated M tb into mice and found it was equally as protective as BCG injected intradermally against aerosol challenge with M tb[37].

**[0028]** Delivery of the irradiated bacteria or the bacterial antigens to the lung and mucosal border is believed to facilitate an effective immune response in the host. Upon delivery to the nasal mucosa or alveolar passages, the bacteria or bacteria antigens are detected by antigen presenting cells, specifically dendritic cells at the alveoli/interstitial space of the lung. These dendritic cells then migrate to the regions enriched in naïve CD4+ and CD8 + T-cells and which constitute the paracortical zone of the regional lymph nodes of the lung. These T cells are activated by the dead bacilli's antigens. The dead mycobacteria will become phagocytosed by macrophages.

**[0029]** In general, any Mycobacterium species or strain that is a member of the M. tuberculosis complex can be used in the composition and methods of the disclosure. Suitable species,

Mycobacterium which are members of the M tb complex include, e.g, Mycobacterium bovis, Mycobacterium africanum, Mycobacterium microtti, and Mycobacterium tuberculosis. Mycobacterium that are genetically similar include Mycobacterium canettii and Mycobacterium marinum. The particular species or combination of species is selected for the corresponding host species and type Mycobacterium- associated disease to be treated. Other Mycobacteria that cause disease in humans include, e.g., Mycobacterium avium intracellulare, Mycobacterium leprae, Mycobacterium lepraemurium, Mycobacteria paratuberculosis, Mycobacterium ulcerans, Mycobacterium smegmatis, , Mycobacterium xenopi, Mycobacterium chelonei, Mycobacterium fortuitum, Mycobacterium farcinogenes, Mycobacterium flavum, Mycobacterium haemophitum, Mycobacterium kansasii, Mycobacterium phlei, Mycobacterium scrofulaccum, Mycobacterium senegalense, Mycobacterium simiae, Mycobacterium thermoresistible, and Mycobacterium xenopi.

**[0030]** The mycobacterium to be used in the pharmaceutical composition include whole cells.

Preparing pharmaceutical compositions

**[0031]** The killed cells are prepared for administration to a host by combining inactivated cells or cell lysates with a pharmaceutically acceptable carrier to form a pharmaceutical composition. The carrier can be, e.g., such as physiological saline, mineral oil, vegetable oils, aqueous sodium carboxymethyl cellulose, or aqueous polyvinylpyrrolidone. In some embodiments, the carrier is sufficiently pure to be administered therapeutically to a human subject. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, or Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be included, as required.

**[0032]** A skilled person in the field familiar with the protocols, formulations, dosages and clinical practice associated with, e.g., the administration of M. bovis BCG can in addition readily adapt these protocols for use with pharmaceutical compositions of the present invention. The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, including, e.g., the capacity of the individual's immune system to mount an immune response, and the degree of protection desired. Suitable dosage ranges are of the order of several hundred micrograms active ingredient per vaccination with a preferred range from about 0.1 μg to 1000 μg, such as in the range from about 1 μg to 300 μg, and especially in the range from about 10 μg to 50 μg. Suitable regimens for initial administration and booster shots are also variable but are typified by an initial administration followed by subsequent inoculations or other administrations. Thus, the vaccine may be administered in a single dose or in a plurality of doses. In one embodiment, the vaccine may be administered in two doses about 1-12 months apart. The subject may be vaccinated at any time, although it is preferred to administer the vaccine shortly (optimally about 10 days to two weeks) before periods of anticipated stress, such as during shipping or other handling. It is also envisioned that the vaccine may be administered to pregnant animals prior to birth to increase production of hyper immune colostrum.

**[0033]** A composition may be administered alone or in combination with other treatments or standard BCG vaccine, either simultaneously or sequentially dependent upon the condition to be treated. The composition can be administered after vaccination with BCG and therefore act as a boosting tuberculosis vaccine. Moreover, it may be given after an initial subcutaneous inoculation of the whole killed bacilli followed by an intranasal or mucosal boost.

**[0034]** The killed cells may be incorporated into microparticles or microcapsules to prolong the exposure of the antigenic material to the subject animal and hence protect the animal against infection for long periods of time. The microparticles and capsules may be formed from a variety of well-known inert, biocompatible matrix materials using techniques conventional in the art. Suitable matrix materials include, e.g., natural or synthetic polymers such as alginates, poly(lactic acid), poly(lactic/glycolic

acid), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyortho esters, polyacetals, polycyanoacrylates, polyurethanes, ethytlene-vinyl acetate copolymers, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolefins, polyethylene oxide, and particularly agar and polyacrylates. Examples of techniques for incorporation of materials into microparticles or encapsulation which may be used herein are described by Sparks[38], Kydonius[39], and El-Nokaly[40].

**[0035]** The inactivated mycobacterium may be contained in small particles suspended in the water or saline. The vaccine formulations may also contain optional adjuvants, antibacterial agents or other pharmaceutically active agents as are conventional in the art. Adjuvants may include but are not limited to salts, emulsions (including oil/water compositions), saponins, liposomal formulations, virus particles, polypeptides, pathogen-associated molecular patterns (PAMPS), nucleic acid-based compounds or other formulations utilizing certain antigens. Suitable adjuvants include, e.g., vegetable oils, alum, Freund's incomplete adjuvant, or Freund's incomplete adjuvant, with oils and Freund's incomplete adjuvant being particularly preferred. Other adjuvants include agents such as aluminum hydroxide or phosphate (alum), immune-stimulating complexes (ISCOMs), synthetic polymers of sugars (CARBOPOL®), aggregation of the protein in the vaccine by heat treatment, aggregation by reactivating with pepsin treated (Fab) antibodies to albumin, mixture with bacterial cells such as C. parvum or endotoxins or lipopolysaccharide components of gram-negative bacteria, emulsion in physiologically acceptable oil vehicles such as mannide mono-oleate (Aracel A) or emulsion with 20 percent solution of a perfluorocarbon (Fluosol-DA) used as a block substitute may also be employed.

**[0036]** The inactivated mycobacterium may be contained in a mucosal bacterial toxin adjuvant such as the *Escherichia coli* labile toxi (LT) and cholera toxin (CT) or in CpG oligodeoxynucleotide (CpG ODN)[41]. Another possible mucosal adjuvant Monophosphoryl lipid A (MPL), a derivative and less toxic form of LPS, when combined with liposomes was found to induce mucosal immunoprotective responses[42]. One new adjuvant designed for nasal vaccination, Eurocine L3™, has been shown to induce long-lasting immunity against TB in experimental animal models after intranasal administration[43-45]. The adjuvant technology consists of a non-toxic pharmaceutical formulation based on a combination of endogenous and pharmaceutically accepted lipids. The vaccine may optionally include additional immune modulating substances such as cytokines or synthetic IFN-γ inducers such as poly I:C alone or in combination with the above-mentioned adjuvants.

**[0037]** Still other adjuvants include microparticles or beads of biocompatible matrix materials. The microparticles may be composed of any biocompatible matrix materials as are conventional in the art, including but not limited to, agar and polyacrylates. The practitioner skilled in the art will recognize that other carriers or adjuvants may be used as well. For example, Chitosan or any bioadhesive delivery system which may be used are described by Webb and Winkelstein [46] the contents of which are incorporated by reference herein.

**[0038]** The pharmaceutical composition containing the inactivated mycobacterium is preferably formulated for intranasal or intrapulmonary delivery using methods known in the art. The formulation of the irradiated mycobacterium combined with the adjuvant is preferably selected to minimize side effects, such as inflammation, associated with vaccination or may improve the formulation's stability. The adjuvant may also have a role as an immunostimulant or as a depot.

**[0039]** In some embodiments, the inactivated mycobacterium are delivered by the refinement of a nebulizer or via three types of compact portable devices, the metered-dose inhaler (MDI) and the dry powder inhaler (DPI). Intransal delivery can occur via the nasal spray, dropper or nasal metered drug delivery device. The inactive mycobacterium may be delivered via a metered dose inhaler. Typically, only 10-20% of the emitted dose is deposited in the lung. The high velocity and large particle size of the spray causes approximately 50-80% of the drug aerosol to impact in the oropharyngeal region.

**[0040]** The mycobacterium may be contained in a dry powder formulation such as but not limited to a sugar carrier system. The Sugar Carrier System could include lactose, mannitol, and/or glucose. Lactose, mannitol, and glucose are all approved by the FDA as carriers. There are also larger sugar particles such as lactose monohydrate- typically 50-100 micrometers in diameter, which remain in the naso-oropharynx but allows the inactivated bacilli to travel through the respiratory tree into the alveoli.[47]

**[0041]** If desired, the mycobacterium may be contained in a liposomal formulation. Liposomes, like other inhaled particles reaching the alveoli, are cleared by macrophages. The processing, uptake and recycling of liposomal phospholipids occurs through the same mechanism as endogenous surfactant via the alveolar type II cells.

**[0042]** A pharmaceutical composition containing the irradiated mycobacterium described above is administered to a suitable individual for preventing or treating tuberculosis. Reference herein to "tuberculosis" includes reference to pulmonary and extra-pulmonary tuberculi. The terms "individual," "subject," "host," and "patient," are used interchangeably herein and refer to any subject having a bacterial infection amenable to treatment using the therapeutic vaccine of the invention, and for whom treatment or therapy is desired. The pharmaceutical composition can be prepared for any mammalian host that is susceptible to infection by mycobacterium. Suitable mammalian hosts include, e.g., farm animals such as swine and bovine

**[0043]** The terms "treatment", "treating", "treat" and the

like are used herein to generally refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete stabilization or cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a subject, particularly a mammalian subject, more particularly a human, and includes: (a) preventing the disease or symptom from occurring in a subject which may be predisposed to the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease symptom, i.e., arresting its development; or relieving the disease symptom, i.e., causing regression of the disease or symptom (c) preventing reactivation of the disease in latent TB, i.e. preventing the bacilli from transitioning from a dormant to growth phase. Thus, administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical or veterinarian.

**[0044]** The subject treated with the vaccine typically will have or will develop protective immunity to an infecting bacterium. The term "protective immunity" means that a vaccine, immunogenic composition or immunization schedule that is administered to a mammal induces an immune response that prevents, retards the development of, or reduces the severity of a disease that is caused by a pathogenic bacterium or diminishes or altogether eliminates the symptoms of the disease. By "infecting bacterium" is meant a bacterium that has established infection in the host, and which may be associated with a disease or undesirable symptom as a result. Generally, infecting bacteria are pathogenic bacteria.

**[0045]** The phrase "in a sufficient amount to elicit an immune response" means that there is a detectable difference between an immune response indicator measured before and after administration of a particular vaccine preparation or immunogenic composition. Animals given the vaccine trial will be tested against animals give intradermal BCG (as the gold standard). Several weeks after the last vaccination, animals will be challenged with aerosol virulent M tb. The clinical and molecular immune response will be evaluated several weeks after challenge with virulent M. tb.

Screening and Developing Tuberculosis Vaccines

**[0046]** A test vaccine can be screened or optimized by subjecting a population of mycobacterium cells, or fractions thereof (as described above) to various inactivation regimens, preparing a candidate pharmaceutical composition containing the treated cells or cell fractions and testing the ability of the treated composition using the methods described above to elicit an immune response and/or mount an effective challenge to mycobacterium infection in a host.

**[0047]** The terms "immunogenic bacterial composition", "immunogenic composition", and "vaccine" are used interchangeably herein to mean a preparation capable of eliciting a cellular and/or humoral immune response in a subject when administered in a sufficient amount to elicit an immune response to epitopes present in said preparation.

**[0048]** Immunopotency of the antigenic molecule expressed by the mycobacterium cell or extract preparation, can be determined by monitoring the immune response of test animals following immunization with the bacteria expressing the recombinant antigen. Test animals may include mice, guinea pigs, rabbits, bovine, non-human primates, and eventually human subjects.

**[0049]** The immune response of the test subject can additionally be analyzed by various approaches such as: (a) T-cell associate cytokine production (b) plasma cytokine production (c) T cell proliferation, cytoxicity, cytokine profiles (d) T cell antigen repertoire (e) T cell regulatory profiles (f) mRNA profiles (g) innate immunity profiles (h) antibody profiles (i) genetics and (j) protection from disease and/or mitigation of infectious symptoms in immunized animals.

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## Claims

1. A pharmaceutical composition comprising inactivated whole *Mycobacterium tuberculosis* (*M. tb*), wherein said composition is formulated for intranasal, mucosal or intrapulmonary delivery to a mammalian host, and wherein said composition comprises an immunologically protective dose when delivered to said host and wherein said *M. tb* is inactivated with irradiation.

2. A composition comprising inactivated whole *M. tb* for use in vaccinating a mammal against Tuberculosis (TB), wherein said vaccination of said mammal is intranasal or intrapulmonary, and wherein said composition comprises an immunologically protective dose when delivered to said host and wherein said *M. tb* is inactivated with irradiation.

3. The composition of claim 1 or the composition for the use according to claim 2, wherein 100 % of said *M. tb* are inactivated.

4. The composition of claim 1 or the composition for the use according to claim 2, wherein said irradiation is gamma radiation.

5. The pharmaceutical composition of claim 1 or the composition for the use according to claim 2, further comprising *Mycobacterium* cell lysates.

6. The composition of claim 1, further comprising an adjuvant to enhance an immune response in said host.

7. The composition of claim 1 or the composition for the use according to claim 2, further comprising a pharmaceutically acceptable carrier.

8. The composition of claim 1, wherein said composition is lyophilized.

9. An aerosol or spray package comprising the pharmaceutical composition of claim 1.

10. The composition of claim 7 or the composition for the use according to claim 7 , wherein said composition is for prophylactic or therapeutic use in said mammal.

11. A method for preparing a pharmaceutical composition, comprising formulating an immunologically protective dose of an inactivated whole *M. tb* for intranasal or pulmonary delivery to a mammalian host and wherein said *M. tb* are inactivated by irradiation.

12. The composition for the use according to claim 2, further comprising an adjuvant to enhance an immune response in said host without causing debilitating inflammation.

13. The composition for the use according to claim 12 , for simultaneous or sequential use with Bacille Calmette-Guerin (BCG).

14. The composition according to any one of claims 1, 3-8 or 10 or the composition for the use according to any one of claims 2-5, 7, 10, 12 or 13 , the aerosol spray package according to claim 9, or the method according to claim 11, wherein the dose of the *M. tb* is from 0.1 to 50 micrograms.

15. The composition according to claims 10 or 14 or the composition for the use according to claims 10, 12 or 13, the aerosol spray package according to claim 9 or 14, or the method according to claim 11 or 14, wherein the composition is provided lyophilized.

16. The composition according to any one of claims 1, 3-8, 10, 14 or 15 or the composition for the use according to any one of claims 2-5, 7, 10, 12, 13, 14 or 15, the aerosol spray package according to any one of claims 9, 14 or 15 or the method according to any one of claims 11, 14 or 15, wherein the mammal is a human.

**Patentansprüche**

1. Pharmazeutische Zusammensetzung, umfassend ganzes inaktiviertes *Mycobacterium tuberculosis* (*M. bb.*), wobei die Zusammensetzung zur intranasalen, mukosalen oder intrapulmonalen Verabreichung an einen Säugerwirt formuliert ist, und wobei die Zusammensetzung eine immunologisch schützende Dosis umfasst, wenn sie dem Wirt verabreicht wird, und wobei das *M. tb.* durch Bestrahlung inaktiviert ist.

2. Zusammensetzung, umfassend ganzes inaktiviertes *M. fb.* zur Verwendung bei der Impfung eines Säugers gegen Tuberkulose (TB), wobei die Impfung des Säugers intranasal oder intrapulmonal ist, und wobei die Zusammensetzung eine immunologisch schützende Dosis umfasst, wenn sie dem Wirt verabreicht wird, und wobei das *M. tb.* durch Bestrahlung inaktiviert ist.

3. Zusammensetzung nach Anspruch 1 oder Zusammensetzung zur Verwendung nach Anspruch 2, wobei 100% des *M. tb.* inaktiviert sind.

4. Zusammensetzung nach Anspruch 1 oder Zusammensetzung zur Verwendung nach Anspruch 2, wobei die Bestrahlung Gammastrahlung ist.

5. Pharmazeutische Zusammensetzung nach Anspruch 1 oder Zusammensetzung zur Verwendung nach Anspruch 2, weiterhin umfassend Lysate von *Mycobacterium-Zellen*.

6. Zusammensetzung nach Anspruch 1, weiterhin umfassend ein Adjuvans zum Verstärken einer Immunantwort in dem Wirt.

7. Zusammensetzung nach Anspruch 1 oder Zusammensetzung zur Verwendung nach Anspruch 2, weiterhin umfassend einen pharmazeutisch verträglichen Träger.

8. Zusammensetzung nach Anspruch 1, wobei die Zusammensetzung lyophilisiert ist.

9. Aerosol- oder Spraypackung, umfassend die pharmazeutische Zusammensetzung nach Anspruch 1.

10. Zusammensetzung nach Anspruch 7 oder Zusammensetzung zur Verwendung nach Anspruch 7, wobei die Zusammensetzung zur vorbeugenden oder therapeutischen Verwendung an dem Säuger ist.

11. Verfahren zur Herstellung einer pharmazeutischen Zusammensetzung, umfassend Formulieren einer immunologisch schützenden Dosis eines ganzen inaktivierten *M. tb.* zur intranasalen oder intrapulmonalen Verabreichung an einen Säugerwirt, und wobei die *M. tb.* durch Bestrahlung inaktiviert sind.

12. Zusammensetzung zur Verwendung nach Anspruch 2, weiterhin umfassend ein Adjuvans zum Verstärken einer Immunantwort in dem Wirt, ohne eine entkräftende Entzündung auszulösen.

13. Zusammensetzung zur Verwendung nach Anspruch 12 zur gleichzeitigen oder zeitlich abgestuften Ver-

wendung mit Bacillus Calmette-Guérin (BCG).

**14.** Zusammensetzung nach einem der Ansprüche 1, 3-8 oder 10 oder Zusammensetzung zur Verwendung nach einem der Ansprüche 2-5, 7, 10, 12 oder 13, Aerosolspraypackung nach Anspruch 9 oder Verfahren nach Anspruch 11, wobei die Dosis des *M. tb.* 0,1 bis 50 Mikrogramm beträgt.

**15.** Zusammensetzung nach den Ansprüchen 10 oder 14 oder Zusammensetzung zur Verwendung nach den Ansprüchen 10, 12 oder 13, Aerosolspraypackung nach Anspruch 9 oder 14 oder Verfahren nach Anspruch 11 oder 14, wobei die Zusammensetzung lyophilisiert bereitgestellt wird.

**16.** Zusammensetzung nach einem der Ansprüche 1, 3-8, 10, 14 oder 15 oder Zusammensetzung zur Verwendung nach einem der Ansprüche 2-5, 7, 10, 12, 13, 14 oder 15, Aerosolspraypackung nach einem der Ansprüche 9, 14 oder 15 oder Verfahren nach einem der Ansprüche 11, 14 oder 15, wobei der Säuger ein Mensch ist.

**Revendications**

**1.** Composition pharmaceutique comprenant *Mycobacterium tuberculosis (M. tb)* entier inactivé, laquelle composition est formulée pour une délivrance par voie intranasale, muqueuse ou intrapulmonaire à un hôte mammifère, laquelle composition comprend une dose immunologiquement protectrice quand elle est délivrée audit hôte, dans laquelle ledit *M. tb* est inactivé par irradiation.

**2.** Composition comprenant *M. tb* entier inactivé pour utilisation dans la vaccination d'un mammifère contre la tuberculose (TB), dans laquelle ladite vaccination dudit mammifère est intranasale ou intrapulmonaire, laquelle composition comprend une dose immunologiquement protectrice quand elle est délivrée audit hôte, dans laquelle ledit *M. tb* est inactivé par irradiation.

**3.** Composition selon la revendication 1 ou composition pour utilisation selon la revendication 2, dans laquelle 100 % dudit *M. tb* sont inactivés.

**4.** Composition selon la revendication 1 ou composition pour utilisation selon la revendication 2, dans laquelle ladite irradiation est un rayonnement gamma.

**5.** Composition selon la revendication 1 ou composition pour utilisation selon la revendication 2, comprenant en outre des lysats cellulaires de *Mycobacterium.*

**6.** Composition selon la revendication 1, comprenant en outre un adjuvant pour amplifier une réponse immunitaire chez ledit hôte.

**7.** Composition selon la revendication 1 ou composition pour utilisation selon la revendication 2, comprenant en outre un véhicule pharmaceutiquement acceptable.

**8.** Composition selon la revendication 1, laquelle composition est lyophilisée.

**9.** Conditionnement en aérosol ou en spray comprenant la composition pharmaceutique selon la revendication 1.

**10.** Composition selon la revendication 7 ou composition pour utilisation selon la revendication 7, laquelle composition est destinée à un usage prophylactique ou thérapeutique chez ledit mammifère.

**11.** Procédé pour préparer une composition pharmaceutique, comprenant la formulation d'une dose immunologiquement protectrice d'un ***M.*** *tb* entier inactivé pour une délivrance par voie intranasale ou pulmonaire à un hôte mammifère, dans lequel ledit *M. tb* est inactivé par irradiation.

**12.** Composition pour utilisation selon la revendication 2, comprenant en outre un adjuvant pour amplifier une réponse immunitaire chez ledit hôte sans provoquer d'inflammation débilitante.

**13.** Composition pour utilisation selon la revendication 12, pour utilisation simultanée ou séquentielle avec le bacille de Calmette-Guérin (BCG).

**14.** Composition selon l'une quelconque des revendications 1, 3 à 8 et 10, ou composition pour utilisation selon l'une quelconque des revendications 2 à 5, 7, 10, 12 et 13, conditionnement en spray aérosol selon la revendication 9, ou procédé selon la revendication 11, dans lequel la dose de *M. tb* est de 0,1 à 50 microgrammes.

**15.** Composition selon la revendications 10 ou 14 ou composition pour utilisation selon la revendication 10, 12 ou 13, conditionnement en spray aérosol selon la revendication 9 ou 14, ou procédé selon la revendication 11 ou 14, dans lequel la composition est fournie à l'état lyophilisé.

**16.** Composition selon l'une quelconque des revendications 1, 3 à 8, 10, 14 et 15, ou composition pour utilisation selon l'une quelconque des revendications 2 à 5, 7, 10, 12, 13, 14 et 15, conditionnement en spray aérosol selon l'une quelconque des revendications 9, 14 et 15, ou procédé selon l'une quelconque des revendications 11, 14 et 15, dans lequel

le mammifère est un humain.

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# TUBERKOLÓZIS-VAKCINA ÉS ELJÁRÁS AZ ALKALMAZÁSÁRA

## Szabadalmi igénypontok

1. Gyógyászati készítmény, amely tartalmaz inaktivált teljes *Mycobacterium tuberculosis*-t (*M. tb*), ahol a készítmény egy emlős gazdába történő intranazális, mukozális vagy intrapulmonáris bejuttatáshoz van formulálva, és ahol a készítmény egy immunológiai védőhatást biztosító dózist tartalmaz amikor a gazdába juttatjuk, és ahol az *M. tb* besugárzással van inaktiválva.

2. Inaktivált teljes *M. tb*-t tartalmazó készítmény egy emlős tuberkolózissal (TB) szembeni vakcinázására, ahol az emlős vakcinázása intranazális vagy intrapulmonális úton történik, és ahol a készítmény egy immunológiai védőhatást biztosító dózist tartalmaz amikor a gazdába juttatjuk, és ahol az *M. tb* besugárzással van inaktiválva.

3. Az 1. igénypont szerinti készítmény vagy készítmény alkalmazásra a 2. igénypont szerint, ahol az *M. tb* 100%-a inaktivált.

4. Az 1. igénypont szerinti készítmény vagy készítmény alkalmazásra a 2. igénypont szerint, ahol a besugárzás gamma sugárzás.

5. Az 1. igénypont szerinti gyógyászati készítmény vagy készítmény alkalmazásra a 2. igénypont szerint, amely *Mycobacterium* sejtekből készített lizátumokat is tartalmaz.

6. Az 1. igénypont szerinti készítmény, amely adjuvánst is tartalmaz annak érdekében, hogy fokozzon egy immunválaszt a gazdában.

7. Az 1. igénypont szerinti készítmény vagy készítmény alkalmazásra a 2. igénypont szerint, amely gyógyászatilag elfogadható hordozót is tartalmaz.

8. Az 1. igénypont szerinti készítmény, ahol a készítmény liofilizálva van.

9. Aeroszol vagy spray formában kiszerelt termék, amely tartalmazza az 1. igénypont szerinti gyógyászati készítményt.

10. A 7. igénypont szerinti készítmény vagy készítmény alkalmazásra a 7. igénypont szerint, ahol a készítmény preventív vagy terápiás célra szolgál az emlősben.

11. Eljárás gyógyászati készítmény előállítására, amely eljárás tartalmazza, hogy egy inaktivált teljes *M. tb* egy immunológiai védőhatást biztosító dózisát egy emlős gazdába történő intranazális vagy pulmonális bejuttatáshoz formuláljuk, és ahol az *M. tb* besugárzással van inaktiválva.

12. Készítmény alkalmazásra a 2. igénypont szerint, amely adjuvánst is tartalmaz annak érdekében, hogy anélkül fokozzon egy immunválaszt a gazdában, hogy legyengülést okozó gyulladást váltana ki.

13. Készítmény alkalmazásra a 12. igénypont szerint Bacille Calmette-Guerin (BCG) baktériummal való egyidejű vagy egymást követő alkalmazásra.

14. Az 1., 3-8. vagy 10. igénypontok bármelyike szerinti készítmény, vagy készítmény alkalmazásra a 2-5., 7., 10., 12. vagy 13. igénypontok bármelyike szerint, a 9. igénypont szerinti, aeroszol vagy spray formában kiszerelt termék, vagy a 11. igénypont szerinti eljárás, ahol az *M. tb* dózisa 0,1 - 50 mikrogramm.

15. Az 10. vagy 14. igénypont szerinti készítmény vagy készítmény alkalmazásra a 10., 12. vagy 13. igénypontok bármelyike szerint, a 9. vagy 14. igénypont szerinti, aeroszol vagy spray formában kiszerelt termék, vagy a 11. vagy 14. igénypontok bármelyike szerinti eljárás, ahol a készítményt liofilizált formában biztosítjuk.

16. Az 1., 3-8., 10., 14. vagy 15. igénypontok bármelyike szerinti készítmény vagy készítmény alkalmazásra a 2-5., 7., 10., 12., 13., 14. vagy 15. igénypontok bármelyike szerint, a 9., 14. vagy 15. igénypontok bármelyike szerinti, aeroszol vagy spray formában kiszerelt termék, vagy a 11., 14. vagy 15. igénypontok bármelyike szerinti eljárás, ahol az emlős egy ember.