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(54) **PROTEINS EXPRESSED BY MYCOBACTERIUM TUBERCULOSIS AND NOT BY BCG AND THEIR USE AS DIAGNOSTIC REAGENTS AND VACCINES**

DURCH MYCOBACTERIUM TUBERCULOSIS UND NICHT DURCH BCG EXPRIMIERTE PROTEINE UND IHRE VERWENDUNG ALS DIAGNOSTISCHE REAGENZIEN UND IMPFSTOFFE

PROTEINES EXPRIMEES PAR MYCOBACTERIUM TUBERCULOSIS ET NON PAR BCG ET EMPLOYEES EN TANT QUE VACCINS ET REACTIFS DE DIAGNOSTIC

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Description

[0001] The invention is in the field of tuberculosis and, specifically, reagents useful for generating immure responses to *Mycobacterium tuberculosis* and for diagnosing infection and disease in a subject that has been exposed to *M. tuberculosis.*

Background of the Invention

[0002] Tuberculosis infection continues to be a worldwide health problem. This situation has recently been greatly exacerbated by the emergence of multi-drug resistant strains of *M. tuberculosis* and the international AIDS epidemic. It has thus become increasingly important that effective vaccines against and reliable diagnostic reagents for *M. tuberculosis* be produced.

Summary of the Invention

[0003] The invention is based on the inventor's discovery that a polypeptide encoded by an open reading frame (ORF) in the genome of *M. tuberculosis* that is absent from the genome of the Bacille Calmette Guerin (BCG) strain of *M. bovis* elicited a delayed-type hypersensitivity response in animals infected with *M. tuberculosis* but not in animals sensitized with BCG. Thus proteins encoded by ORFs present in the genome of *M. tuberculosis* but absent from the genome of BCG represent reagents that are useful in discriminating between *M. tuberculosis* and BCG and, in particular, for diagnostic methods (e.g., skin tests and *in vitro* assays for *M. tuberculosis*-specific antibodies and lymphocyte responsiveness) which discriminate between exposure of a subject to *M. tuberculosis* and vaccination with BCG. Thus, the present invention relates to *in vitro* methods of diagnosis which discriminate between exposure of a subject to *M. tuberculosis* and exposure of a subject to BCG. In one embodiment, the method comprises testing a population of cells from a subject for the presence of CD4 T Lymphocytes that respond to MTBN4, as depicted in Figure 1, wherein the presence of CD4 T lymphocytes that respond to MTBN4 indicates that the subject has been exposed to *M. tuberculosis* and not to BCG without exposure to *M. tuberculosis.*

[0004] In an alternative embodiment, the method comprises testing a subject for the presence of B lymphocytes which produce antibodies that bind to MTBN4, the testing being carried out on a bodily fluid from the subject, wherein the presence in the subject of B lymphocytes that produce antibodies that bind to MTBN4 indicates that the subject has been Infected by exposure to *M. tuberculosis* and not to BCG without exposure to *M. tuberculosis.*

[0005] The invention also features vectors comprising the DNA molecules encoding polypeptides MTBN4 and compositions comprising these polypeptides. A variety of diagnostic methodologies utilizing the foregoing are also provided.

[0006] In one embodiment, the invention features a vector comprising: (a) the DNA molecule encoding the polypeptide MTBN4, as depicted in Figure 1, and (b) transcriptional and translational regulatory sequences operationally linked to said DNA sequence, said regulatory sequences allowing for expression of the polypeptide encoded by said DNA sequence In a cell, for use as a diagnostic. The encoded polypeptide has *Mycobacterium tuberculosis* specific antigenic and immunogenic properties. Also defined herein is an isolated portion of the above DNA molecule. The portion of the DNA molecule encodes a segment of the polypeptide shorter than the full-length polypeptide, and the segment has *Mycobacterium tuberculosis* specific antigenic and immunogenic properties. Cells which can be used to express the polypeptide encoded within the vector include both eukaryotic and prokaryotic cells.

[0007] The above vectors can be incorporated into compositions comprising a pharmaceutically acceptable diluent or filler. Other compositions which can be used contain at least two (eg three, four, five, six, seven, eight, nine, ten, twelve, fifteen, or twenty) DNA sequences, each encoding a polypeptide of the *Mycobacterium tuberculosis* complex or a functional segment thereof, with the DNA sequences being operationally linked to transcriptional and translational regulatory sequences which allow for expression of each of the polypeptides in a cell of a vertebrate. In such compositions, at least one (eg two, three, four, five, six, seven or eight) of the DNA sequences is a DNA molecule encoding the polypeptide MTBN4, as depicted in Figure 1. The encoded polypeptides will preferably be those not encoded by the genome of cells of the BCG strain of *M. bovis.*

[0008] The specification describes a polypeptide comprising the MTBN4 sequence as depicted in Fig. 1. The invention also features a composition comprising said polypeptide and at least one other polypeptide of the *Mycobacterium tuberculosis* complex, not being encoded by the genome of the cells of the BCG strain of *Mycobacterium bovis,* for use as a diagnostic. The at least one other polypeptide can be selected from the group consisting of MTBN1, MTBN2, MTBN3, MTBN4, MTBN5, MTBN6, MTBN7 and MTBN8 as depicted in Figure 1, or polypeptides with amino acid sequences identical thereto with conservative substitutions. The polypeptide has *Mycobacterium tuberculosis* specific antigenic and immunogenic properties. Also defined herein is an isolated segment of the above polypeptides, the segment being shorter than the full-length polypeptide and having *Mycobacterium tuberculosis* specific antigenic and immunogenic properties. The polypeptides and composition of the invention can be used with a pharmaceutically acceptable diluent or filler. Compositions of the invention can also contain at least two (eg three, four, five, six, seven, eight, nine, ten, twelve, fifteen, or twenty) polypeptides of the *Mycobacterium tuberculosis* complex, or functional segments thereof, with at least one of the at least two (eg two, three, four, five, six, seven, or eight)

polypeptides comprising the MTBN4 sequence, as depicted in figure 1. The polypeptides will preferably be those not encoded by the genome of cells of the BCG strain of *M. bovis.*

[0009] The invention also features polypeptides, compositions and vectors as defined above, for use as diagnostics. Although not part of the invention as such, methods can be employed involving (a) administration of one of the above polypeptide compositions to a subject suspected of having or being susceptible to *Mycobacterium tuberculosis* infection; and (b) detecting an immune response in the subject to the composition, as an indication that the subject has or is susceptible to *Mycobacterium tuberculosis* infection. An example of such a method is a skin test in which the test substance (e.g., compositions containing one or more of MTBN1 - MTBN8) is injected intradermally into the subject and in which a skin delayed-type hypersensitivity response is tested for.

[0010] In a further aspect, the invention relates to an *in vitro* method of diagnosis comprising (a) contacting CD4 T lymphocytes with antigen presenting cells (APC) in the presence of a polypeptide comprising the MTBN4 sequence, as depicted in Figure 1, wherein said CD4 T lymphocytes are from a population of cells from a subject, and wherein said APC express at least one major histocompatibility complex (MHC) class II molecule expressed by said subject; and (b) determining the ability of said CD4 T lymphocytes to respond to said polypeptide, as an indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection. Another *in vitro* diagnostic method of the invention involves: (a) contacting a polypeptide comprising the MTBN4 sequence, as depicted in Figure 1 with a bodily fluid of a subject; (b) detecting the presence of binding of antibody to said polypeptide as indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection.

[0011] In the above *in vitro* diagnostic methods of the invention, the polypeptide comprising MTBN4 may be present within a composition comprising at least one other polypeptide as defined above.

[0012] Also encompassed by the invention is the use of a polypeptide composition or vector as defined herein, for the manufacture of a medicament for diagnosing exposure of a subject to *Mycobacterium tuberculosis.*

[0013] As used herein, an "isolated DNA molecule" is a DNA which is one or both of: not immediately contiguous with one or both of the coding sequences with which it is immediately contiguous (i.e.. one at the 5' end and one at the 3' end) in the naturally-occurring genome of the organism from which the DNA is derived; or which is substantially fee of DNA sequence with which it occurs in the organism from which the DNA is derived. The term includes, for example, a recombinant DNA which incorporated into a vector, e.g., into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic fragment produced by PCR or restriction endonuclease treatment) independent of other DNA sequences. Isolated DNA also includes a recombinant DNA which is part of a hybrid DNA encoding additional *M. tuberculosis* polypeptide sequences.

[0014] "DNA molecules" include cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. Where single-stranded, the DNA molecule may be a sense strand or an antisense strand.

[0015] An "isolated polypeptide" of the invention is a polypeptide which either has no naturally-occurring counterpart, or has been separated or purified from components which naturally accompany it, e.g., in *M. tuberculosis* bacteria. Typically, the polypeptide is considered "isolated" when it is at least 70%, by dry weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, a preparation of a polypeptide of the invention is at least 80%, more preferably at least 90%, and most preferably at least 99%, by dry weight, the peptide of the invention. Since a polypeptide that is chemically synthesized is, by its nature, separated from the components that naturally accompany it, the synthetic polypeptide is "isolated."

[0016] An isolated polypeptide of the invention can be obtained, for example, by extraction from a natural source (e.g., *M. tuberculosis* bacteria); by expression of a recombinant nucleic acid encoding the polypeptide; or by chemical synthesis. A polypeptide that is produced in a cellular system different from the source from which it naturally originates is "isolated," because it will be separated from components which naturally accompany it. The extent of isolation or purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

[0017] The polypeptides may contain a primary amino acid sequence that has been modified from those disclosed herein. Preferably these modifications consist of conservative amino acid substitutions. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine.

[0018] The terms "protein" and "polypeptide" are used herein to describe any chain of amino acids, regardless of length or post-translational modification (for example, glycosylation or phosphorylation). Thus, the term *"Mycobacterium tuberculosis* polypeptide" includes full-length, naturally occurring *Mycobacterium tuberculosis* protein, as well a recombinantly or synthetically produced polypeptide that corresponds to a full-length naturally occurring *Mycobacterium tuberculosis* protein or to particular domains or portions of a naturally occurring protein. The term also encompasses a mature *Mycobacterium tuberculosis* polypeptide which has an added amino-terminal methionine (useful for expression in prokaryotic cells) or any short amino acid sequences useful for protein purification by affinity chromatography, e.g., polyhistidine for purification by metal chelate chromatography.

[0019] As used herein, "immunogenic" means capable of activating a primary or memory immune response. Immune responses include responses of CD4+ and CD8+ T lymphocytes and B-lymphocytes. In the case of T lymphocytes, such responses can be proliferative, and/or cytokine (e.g., interleukin(IL)-2, IL-3, IL-4, IL-5, IL-6, IL-12, IL-13, IL-15, tumor necrosis factor-α (TNF-α), or interferon-γ (IFN-γ))-producing, or they can result in generation of cytotoxic T-lymphocytes (CTL). B-lymphocyte responses can be those resulting in antibody production by the responding B lymphocytes.

[0020] As used herein, "antigenic" means capable of being recognized by either antibody molecules or antigen-specific T cell receptors (TCR) on activated effector T cells (e.g., cytokine-producing T cells or CTL).

[0021] Thus, polypeptides that have *"Mycobacterium tuberculosis* specific antigenic properties" are polypeptides that: (a) can be recognized by and bind to antibodies elicited in response to *Mycobacterium tuberculosis* organisms or wild-type *Mycobacterium tuberculosis* molecules (e.g., polypeptides); or (b) contain subsequences which, subsequent to processing of the polypeptide by appropriate antigen presenting cells (APC) and bound to appropriate major histocompatibility complex (MHC) molecules, are recognized by and bind to TCR on effector T cells elicited in response to *Mycobacterium tuberculosis* organisms or wild-type *Mycobacterium tuberculosis* molecules (e.g., polypeptides).

[0022] As used herein, polypeptides that have "*Mycobacterium tuberculosis* specific immunogenic properties" are polypeptides that: (a) can elicit the production of antibodies that recognize and bind to *Mycobacterium tuberculosis* organisms or wild-type *Mycobacterium tuberculosis* molecules (e.g., polypeptides); or (b) contain subsequences which, subsequent to processing of the polypeptide by appropriate antigen presenting cells (APC) and bound to appropriate major histocompatibility complex (MHC) molecules on the surface of the APC, activate T cells with TCR that recognize and bind to peptide fragments derived by processing by APC of *Mycobacterium tuberculosis* organisms or wild-type *Mycobacterium tuberculosis* molecules (e.g., polypeptides) and bound to MHC molecules on the surface of the APC. The immune responses elicited in response to the immunogenic polypeptides are preferably protective. As used herein, "protective" means preventing establishment of an infection or onset of a disease or lessening the severity of a disease existing in a subject. "Preventing" can include delaying onset, as well as partially or completely blocking progress of the disease.

[0023] As used herein, a "functional segment of a *Mycobacterium tuberculosis* polypeptide" is a segment of the polypeptide that has *Mycobacterium tuberculosis* specific antigenic and immunogenic properties.

[0024] Where a polypeptide, functional segment of a polypeptide, or a mixture of polypeptides and/or functional segments have been administered (e.g., by intradermal injection) to a subject for the purpose of testing for a *M. tuberculosis* infection or susceptibility to such an infection, "detecting an immune response" means examining the subject for signs of a immunological reaction to the administered material, e.g., reddening or swelling of the skin at the site of an intradermal injection. Where the subject has antibodies to the administered material, the response will generally be rapid, e.g., 1 minute to 24 hours. On the other hand, a memory or activated T cell reaction of pre-immunized T lymphocytes in the subject is generally slower, appearing only after 24 hours and being maximal at 24-96 hours.

[0025] As used herein, a "subject" can be a human subject or a non-human mammal such as a non-human primate, a horse, a bovine animal, a pig, a sheep, a goat, a dog, a cat, a rabbit, a guinea pig, a hamster, a rat, or a mouse.

[0026] 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 pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention. Unless otherwise indicated, these materials and methods are illustrative only and are not intended to be limiting. All publications, patent applications, patents and other references mentioned herein are illustrative only and not intended to be limiting.

[0027] Other features and advantages of the invention, e.g., methods of diagnosing *M. tuberculosis* infection, will be apparent from the following description, from the drawings and from the claims.

Brief Description of the Drawings

[0028]

Figure 1 is a depiction of the amino acid sequences of *M. tuberculosis* polypeptides MTBN1-MTBN8.

Figure 2 is a depiction of the nucleotide sequences of the coding regions (mtbnl-mtbn8) encoding MTBN1-MTBN8.

Figure 3 is a bar graph showing the delayed-type hypersensitivity responses induced by intradermal injection of 3 different test reagents in female guinea pigs that had been either infected with *M. tuberculosis* cells or sensitized with BCG or *M. avium* cells.

Detailed Description

[0029] The genome of *M. tuberculosis* [Cole et al. (1998) Nature 393:537-544] contains open reading frames (ORFs) that have been deleted from the avirulent BCG strain. The polypeptides encoded by these ORFs are designated herein "*M. tuberculosis* BCG Negative" polypeptides ("MTBN") and the ORFs are designated "mtbn." The invention is based on the discovery that a

MTBN polypeptide (MTBN4) elicited a skin response in animals infected with *M. tuberculosis,* but not in animals sensitized to either BCG or *M. avium,* a non-*M. tuberculosis*-complex strain of mycobacteria (see Example 1 below). These findings indicate that MTBN (e.g., MTBN1-MTBN8) can be used in diagnostic tests that discriminate infection of a subject by *M. tuberculosis* from exposure to both mycobacteria other than the M. tuberculosis-complex and BCG. The *M. tuberculosis*-complex includes *M. tuberculosis, M. bovis, M. microti,* and *M. africanum*. Thus they can be used to discriminate subjects exposed to *M. tuberculosis,* and thus potentially having or being in danger of having tuberculosis, from subjects that have been vaccinated with BCG, the most widely used tuberculosis vaccine. Diagnostic assays that are capable of such discrimination represent a major advance that will greatly reduce wasted effort and consequent costs resulting from further diagnostic tests and/or therapeutic procedures in subjects that have given positive results in less discriminatory diagnostic tests. Furthermore, the results in Example 1 show that MTBN4, as expressed by whole viable *M. tuberculosis* organisms, is capable of inducing a strong immune response in subjects infected with the organisms and thus has the potential to be a vaccine.

[0030] The MTBN polypeptides defined herein include, for example, polypeptides encoded within the RD1, RD2, and RD3 regions of the M. *tuberculosis* genome [Mahairas et al. (1996) J. Bacteriol. 178:1274-1282]. Of particular interest are polypeptides encoded by ORFs within the RD1 regions of the *M. tuberculosis* genome. The amino acid sequences of MTBN1-MTBN8 are shown in Fig. 1 and the nucleotide sequences of mtbn1-mtbn8 are shown in Fig. 2.

[0031] Described herein are: (a) isolated DNA molecules containing mtbn sequences (e.g., mtbn1-mtbn8) encoding MTBN polypeptides (e.g., MTBN1-MTBN8) and isolated portions of such DNA molecules that encode polypeptide segments having antigenic and immunogenic properties (i.e., functional segments); (b) the MTBN polypeptides themselves (e.g., MTBN1-MTBN8) and functional segments of them; (c) antibodies (including antigen binding fragments, e.g., $F(ab')_2$, Fab, Fv, and single chain Fv fragments of such antibodies) that bind to the MTBN polypeptides (e.g., MTBN1-MTBN8) and functional segments; (d) nucleic acid molecules (*e.g.*, vectors) containing and capable of expressing one or more of the mtbn (e.g., mtbn1-mtbn8) sequences and portions of DNA molecules; (e) cells (e.g., bacterial, yeast, insect, or mammalian cells) transformed by such vectors; (f) compositions containing vectors encoding one or more *M. tuberculosis* polypeptides (or functional segments) including both the MTBN (e,g., MTBN1-MTBN8) polypeptides (or functional segments thereof) and previously described *M. tuberculosis* polypeptides such as ESAT-6, 14 kDa antigen, MPT63, 19 kDa antigen, MPT64, MPT51, MTC28, 38 kDa antigen, 45/47 kDa antigen, MPB70, Ag85 complex, MPT53, and KatG (see also U.S. application no. 08/796,792); (g) compositions containing one or more *M. tuberculosis* polypeptides (or functional segments), including both the polypeptides of the invention and previously described *M. tuberculosis* polypeptides such as those described above; (h) compositions containing one or more of the antibodies described in (c); (i) methods of diagnosis involving either (1) administration (e.g., intradermal injection) of any of the above polypeptide compositions to a subject suspected of having or being susceptible to *M. tuberculosis* infection, (2) *in vitro* testing of lymphocytes (B-lymphocytes, CD4 T lymphocytes, and CD8 T lymphocytes) from such a subject for responsiveness (e.g., by measuring cell proliferation, antibody production, cytokine production, or CTL activity) to any of the above polypeptide compositions, (3) testing of a bodily fluid (e.g., blood, saliva, plasma, serum, urine, or semen or a lavage such as a bronchoalveolar lavage, a vaginal lavage, or lower gastrointestinal lavage) for antibodies to the MTBN polypeptides (e.g., MTBN1-MTBN8) or functional segments thereof, or the above-described polypeptide compositions; (4) testing of a bodily fluid (e.g., as above) for the presence of *M. tuberculosis,* MTBN (e.g., MTBN1-MTBN8) polypeptides or functional segments thereof, or the above-described polypeptide compositions in assays using the antibodies described in (c); and (5) testing of a tissue (e.g., lung or bronchial tissue) or a body fluid (e.g., as above) for the presence of nucleic acid molecules (e.g., DNA or RNA) encoding MTBN polypeptides (e.g., MTBN1-MTBN8) (or portions of such a nucleic acid molecules) using nucleic acid probes or primers having nucleotide sequences of the nucleic molecules, portions of the nucleic molecules, or the complements of such molecules; and (j) methods of vaccination involving administration to a subject of the compositions of either (f), (g), (h) or a combination of any two or even all 3 compositions.

[0032] With respect to diagnosis, purified MTBN proteins, functional segments of such proteins, or mixtures of proteins and/or the functional fragments have the above-described advantages of discriminating infection by *M. tuberculosis* from either infection by other bacteria, and in particular, non-pathogenic mycobacteria, or from exposure (by, for example, vaccination) to BCG. Furthermore, compositions containing the proteins, functional segments of the proteins, or mixtures of the proteins and/or the functional segments allows for improved quality control since "batch-to-batch" variability is greatly reduced in comparison to complex mixtures such as purified protein derivative (PPD) of tuberculin.

[0033] The use of the above-described polypeptide and nucleic acid reagents for vaccination also provides for highly specific and effective immunization. Since the virulent *M. tuberculosis* polypeptides encoded by genes absent from avirulent BCG are likely to be mediators of virulence, immunity directed to them can be especially potent in terms of protective capacity. Where vaccination is performed with nucleic acids both *in vivo* and *ex vivo*

methods can be used. *In vivo* methods involve administration of the nucleic acids themselves to the subject and ex *vivo* methods involve obtaining cells (e.g., bone marrow cells or fibroblasts) from the subject, transducing the cells with the nucleic acids, preferably selecting or enriching for successfully transduced cells, and administering the transduced cells to the subject. Alternatively, the cells that are transduced and administered to the subject can be derived from another subject. Methods of vaccination and diagnosis are described in greater detail in U.S. application no. 08/796,792.

[0034] The following example is meant to illustrate, not limit the invention.

Example 1. MPBN4 Elicits a Specific skin Reaction in Guinea Pigs Infected with *M. tuberculosis*

[0035] Four groups of outbred female guinea pigs (18 per group) were used to test the usefulness of the MTBN4 polypeptide as a *M. tuberculosis*-specific diagnostic reagents. The four groups were treated as follows.

Group 1 animals were infected by aerosol with approximately 100 *M. tuberculosis strain H37Rv* cells.

Group 2 animals were sensitized intradermally with 10^6 live *M. bovis* BCG Japanese cells.

Group 3 animals were sensitized intradermally with 10^6 live *M. avium* cells.

Group 4 animals were mock-sensitized by intradermal injection with saline.

[0036] Seven weeks after infection or sensitization, the animals were injected intradermally with 1 μg of PPD (6 animals from each group), 2 μg of purified recombinant MPT64 (6 animals from each group), or 2 ug of MTBN4 (6 animals from each group). The diameter of the resulting erythema was measured 24 hours later. Data are expressed as mean diameter of erythema (in mm) and standard deviations are indicated (Fig. 3).

[0037] No erythema was detected in the group 4 animals with any test substance and thus no data are shown for this group. On the other hand, group 1 animals (solid bars) showed a significant response with all three test substance. Group 2 animals (open bars) showed a significant response to PPD and MPT64 but not MTBN4. Group 3 animals showed a significant response to PPD only (hatched bars).

[0038] Thus, PPD which contains antigenic/immunogenic molecules common to the *M. tuberculosis*-complex as well as other mycobacterial strains, gave the least discriminatory results in that it induced responses in animals infected with or sensitized to mycobacteria of the M. tuberculosis-complex (*M. tuberculosis* and BCG) as well as another non-pathogenic mycobacterium (M. avium). While MPT64, which is encoded and expressed by both *M. tuberculosis* and BCG, did not elicit a response in animals infected with *M. avium,* it did elicit responses in both the *M. tuberculosis* infected and the BCG sensitized animals. Finally, MTBN4 elicited a response in only the *M. tuberculosis* animals. Thus it induced the most specific response and, most importantly, allowed for discrimination between animals infected with *M. tuberculosis* and those sensitized to BCG.

[0039] Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made. Accordingly, the invention is limited only by the following claims.

Claims

1. An *in vitro* method of diagnosis which discriminates between exposure of a subject to *Mycobacterium tuberculosis* (*M. tuberculosis*) and exposure of a subject to the Bacille Calmette Guerin strain of *Mycobacterium bovis* (BCG), the method comprising testing a population of cells from a subject for the presence of CD4 T lymphocytes that respond to MTBN4, as depicted in Figure 1, wherein the presence of CD4 T lymphocytes that respond to MTBN4 indicates that the subject has been exposed to *M. tuberculosis* and not to BCG without exposure to *M. tuberculosis.*

2. An *in vitro* method of diagnosis which discriminates between exposure of a subject to *M. tuberculosis* and exposure of a subject to BCG, the method comprising: testing a subject for the presence of B lymphocytes which produce antibodies that bind to MTBN4, the testing being carried out on a bodily fluid from the subject, wherein the presence in the subject of B lymphocytes that produce antibodies that bind to MTBN4 indicates that the subject has been infected by exposed to *M. tuberculosis* and not to BCG without exposure to *M. tuberculosis.*

3. A method as claimed in claim 1 or claim 2, wherein said polypeptide is present within a composition comprising at least one other polypeptide of the *M. tuberculosis* complex, said at least one other polypeptide not being encoded by the genome of the cells of the BCG strain of *Mycobacterium bovis.*

4. The method of claim 1, wherein the testing of the population of cells from the subject for the presence in the subject of CD4 T lymphocytes that respond to MTBN4 comprises contacting CD4 T lymphocytes from the subject with antigen presenting cells (APC) and MTBN4 or one or more antigenic fragments thereof.

5. The method of claim 1, wherein the testing of the population of cells from the subject for the presence in the subject of CD4 T lymphocytes that respond to MTBN4 comprises testing for cytokine production.

6. The method of claim 5, wherein the cytokine meas-

ured is IFNγ.

7. The method of claim 1, wherein testing a population of cells from a subject for the presence of CD4 T lymphocytes that respond to MTBN4 comprises:

   (a) contacting CD4 T lymphocytes with antigen presenting cells (APC) in the presence of MTBN4 or one or more antigenic fragments thereof, wherein said CD4 T lymphocytes are from a population of cells from a subject, and wherein said APC express at least one major histocompatability complex (MHC) class II molecule expressed by said subject; and
   (b) determining the ability of said CD4 T lymphocytes to respond to MTBN4 or one or more antigenic fragments thereof.

8. The method of claim 2, wherein testing for the presence in the subject of B lymphocytes that produce antibodies that bind to MTBN4, comprises:

   (a) contacting the bodily fluid from the subject with MTBN4 or one or more antigenic fragments thereof; and
   (b) testing for binding of antibody in the body fluid to MTBN4, or the one or more antigenic fragments thereof.

9. The method of claim 8, wherein the bodily fluid is blood.

10. The method of claim 8, wherein the bodily fluid is plasma or serum.

11. A composition comprising at least two polypeptides of the M. *tuberculosis* complex, said at least two polypeptides consisting of the MTBN4 sequence, as depicted in Figure 1, and
    at least one other polypeptide of the *M. tuberculosis* complex, which is not encoded by the genome of the cells of BCG strain of M. *bovis.*

12. A vector comprising:

    (a) the DNA molecule encoding the polypeptide MTBN4, as depicted in Figure 1; and
    (b) transcriptional and translational regulatory sequences linked to said DNA sequence, said regulatory sequences allowing for expression of the polypeptide encoded by said DNA sequence in a cell,

    for use as a diagnostic.

13. The use of a polypeptide comprising the MTBN4 sequence, as depicted in Figure 1, a composition as defined in claim 11, or a vector as defined in claim 12, for the manufacture of a medicament for diagnosing exposure of a subject to *M. tuberculosis.*

Patentansprüche

1. Ein *in vitro*-Vertahren zur Diagnose, welches zwischen der Exposition eines Subjekts an *Mycobacterium tuberculosis* (*M. tuberculosis*) und der Exposition eines Subjekts an den Bacille Calmette Guerin-Stamm von *Mycobacterium bovis* (BCG) unterscheidet, wobei das Verfahren das Testen einer Population von Zellen aus einem Subjekt auf das Vorliegen von CD4 T-Lymphozyten, die auf MTBN4, wie es in Figur 1 dargestellt ist, ansprechen, umfasst, wobei das Vorliegen von CD4 T-Lymphozyten, die auf MTBN4 ansprechen, anzeigt, dass das Subjekt an *M. tuberculosis* und nicht an BCG ohne Exposition an *M. tuberculosis* exportiert wurde.

2. Ein *in vitro*-Verfahren zur Diagnose, welches zwischen der Exposition eines Subjekts an *M. tuberculosis* und der Exposition eines Subjekts an BCG unterscheidet, wobei das Verfahren umfasst: das Testen eines Subjekts auf das Vorliegen von B-Lymphozyten, die Antikörper produzieren, die an MTBN4 binden, wobei das Testen mit einer Körperflüssigkeit aus dem Subjekt durchgeführt wird, wobei das Vorliegen in dem Subjekt von B-Lymphozyten, die Antikörper produzieren, die an MTBN4 binden, anzeigt, dass das Subjekt durch Exposition an *M. tuberculosis* und nicht an BCG ohne Exposition an *M. tuberculosis* infiziert wurde.

3. Ein Verfahren wie in Anspruch 1 oder Anspruch 2 beansprucht, wobei das Polypeptid in einer Zusammensetzung vorliegt, die wenigstens ein anderes Polypeptid des *M. tuberculosis*-Komplexes umfasst, wobei das wenigstens eine andere Polypeptid nicht von dem Genom der Zellen des BCG-Stamms von *Mycobacterium bovis* codiert wird.

4. Das Verfahren nach Anspruch 1, wobei das Testen der Population von Zellen aus dem Subjekt auf das Vorliegen in dem Subjekt von CD4 T-Lymphozyten, die auf MTBN4 ansprechen, das Inkontaktbringen von CD4 T-Lymphozyten aus dem Subjekt mit Antigen präsentierenden Zellen (APC) und MTBN4 oder einem oder mehreren antigenen Fragmenten davon umfasst.

5. Das Verfahren nach Anspruch 1, wobei das Testen der Population von Zellen aus dem Subjekt auf das Vorliegen in dem Subjekt von CD4 T-Lymphozyten, die auf MTBN4 ansprechen, das Testen auf eine Cytokinproduktion umfasst.

6. Das Verfahren nach Anspruch 5, wobei das gemes-

sene Cytokin IFNγ ist.

7. Das Verfahren nach Anspruch 1, wobei das Testen einer Population von Zellen aus einem Subjekt auf das Vorliegen von CD4 T-Lymphozyten, die auf MTBN4 ansprechen, umfasst:

(a) das Inkontaktbringen von CD4 T-Lymphozyten mit Antigen präsentierenden Zellen (APC) in der Gegenwart von MTBN4 oder einem oder mehreren antigenen Fragmenten davon, wobei die CD4 T-Lymphozyten aus einer Population von Zellen aus einem Subjekt stammen und wobei die APC wenigstens ein Haupthistokompatibilitätskomplex (MHC) Klasse II-Molekül exprimieren, das von dem Subjekt exprimiert wird; und
(b) das Bestimmen der Fähigkeit der CD4 T-Lymphozyten, auf MTBN4 oder eines oder mehrere antigene Fragmente davon anzusprechen.

8. Das Verfahren nach Anspruch 2, wobei das Testen auf das Vorliegen in dem Subjekt von B-Lymphozyten, die Antikörper produzieren, die an MTBN4 binden, umfasst:

(a) das Inkontaktbringen der Körperflüssigkeit aus dem Subjekt mit MTBN4 oder einem oder mehreren antigenen Fragmenten davon; und
(b) das Testen auf eine Bindung von Antikörpern in der Körperflüssigkeit an MTBN4 oder das eine oder die mehreren Fragmente davon.

9. Das Verfahren nach Anspruch 8, wobei die Körperflüssigkeit Blut ist.

10. Das Verfahren nach Anspruch 8, wobei die Körperflüssigkeit Plasma oder Serum ist.

11. Eine Zusammensetzung, welche wenigstens zwei Polypeptide des *M. tuberculosis*-Kompexes umfasst, wobei die wenigstens zwei Polypeptide aus der MTBN4-Sequenz, wie sie in Figur 1 dargestellt ist, und
wenigstens einem anderen Polypeptid des *M. tuberculosis*-Komplexes, welches nicht von dem Genom der Zellen des BCG-Stamms von *M. bovis* codiert wird, bestehen.

12. Ein Vektor, welcher umfasst:

(a) das DNA-Molekül, welches das Polypeptid MTBN4, wie es in Figur 1 dargestellt ist, codiert; und
(b) transkriptionale und translationale regulatorische Sequenzen, die mit der DNA-Sequenz verknüpft sind, wobei die regulatorischen Sequenzen eine Expression des Polypeptids, das von der DNA-Sequenz codiert wird, in einer Zelle erlauben,

zur Verwendung als ein Diagnostikum.

13. Die Verwendung eines Polypeptids, welches die MTBN4-Sequenz, wie sie in Figur 1 dargestellt wird, umfasst, einer Zusammensetzung, wie sie in Anspruch 11 definiert wird, oder eines Vektors, wie er in Anspruch 12 definiert wird, zur Herstellung eines Medikaments zum Diagnostizieren der Exposition eines Subjekts an *M. tuberculosis.*

Revendications

1. Procédé de diagnostic *in vitro* qui fait une distinction entre l'exposition d'un sujet à *Mycobacterium tuberculosis* (*M. tuberculosis*) et l'exposition d'un sujet à la souche du Bacille de Calmette Guérin de *Mycobacterium bovis* (BCG), le procédé comprenant l'analyse d'une population de cellules provenant d'un sujet en ce qui concerne la présence de lymphocytes T CD4 répondant à MTBN4, comme décrit dans la Figure 1, analyse selon laquelle la présence de lymphocytes T CD4 répondant à MTBN4 indique que le sujet a été exposé à *M. tuberculosis* et pas au BCG sans exposition à *M. tuberculosis.*

2. Procédé de diagnostic *in vitro* qui fait une distinctioj entre l'exposition d'un sujet à *M. tuberculosis* et l'exposition d'un sujet au BCG, le procédé comprenant : l'analyse d'un sujet en ce qui concerne la présence de lymphocytes B qui produisent des anticorps se liant à MTBN4, l'analyse étant effectuée sur un fluide corporel provenant du sujet, analyse selon laquelle la présence chez le sujet de lymphocytes B qui produisent des anticorps se liant à MTBN4 indiquent que le sujet a été infecté par une exposition à *M. tuberculosis* et pas au BCG sans exposition à *M. tuberculosis.*

3. Procédé selon la revendication 1 ou la revendication 2, dans lequel ledit polypeptide est présent dans une composition comprenant au moins un autre polypeptide du complexe de *M. tuberculosis,* ledit au moins un autre polypeptide n'étant pas codé par le génome des cellules de la souche du BCG de *Mycobacterium bovis.*

4. Procédé selon la revendication 1, dans lequel l'analyse de la population de cellules provenant du sujet en ce qui concerne la présence chez le sujet de lymphocytes T CD4 répondant à MTBN4 comprend la mise en contact de lymphocytes T CD4 provenant du sujet avec des cellules présentatrices d'antigène (APC) et MTBN4 ou un ou plusieurs fragments antigèniques de celui-ci.

5. Procédé selon la revendication 1, dans lequel l'analyse de la population de cellules provenant du sujet en ce qui concerne la présence chez le sujet de lymphocytes T CD4 répondant à MTBN4 comprend une analyse de la production de cytokine.

6. Procédé selon la revendication 5, dans lequel la cytokine mesurée est IFN-γ.

7. Procédé selon la revendication 1, dans lequel une analyse d'une population de cellules provenant d'un sujet en ce qui concerne la présence de lymphocytes T CD4 répondant à MTBN4 comprend :

(a) la mise en contact de lymphocytes T CD4 avec des cellules présentatrices d'antigène (APC) en présence de MTBN4 ou un ou plusieurs fragments antigèniques de celui-ci, dans laquelle lesdits lymphocytes T CD4 proviennent d'une population de cellules provenant d'un sujet, et dans laquelle lesdites APC expriment au moins une molécule de classe II du complexe majeur d'histocompatibilité (MHC) exprimé par ledit sujet ; et
(b) la détermination de la capacité desdits lymphocytes T CD4 à répondre au MTBN4 ou à un ou plusieurs fragments antigèniques de celui-ci.

8. Procédé selon la revendication 2, dans lequel l'analyse en ce qui concerne la présence chez le sujet de lymphocytes B qui produisent des anticorps se liant à MTBN4, comprend :

(a) la mise en contact du fluide corporel provenant du sujet avec MTBN4 ou un ou plusieurs fragments antigèniques de celui-ci ; et
(b) l'analyse de la liaison de l'anticorps du fluide corporel à MTBN4, ou à un ou plusieurs fragments antigèniques de celui-ci.

9. Procédé selon la revendication 8, dans lequel le fluide corporel est du sang.

10. Procédé selon la revendication 8, dans lequel le fluide corporel est du plasma ou du sérum.

11. Composition comprenant au moins deux polypeptides du complexe de *M. tuberculosis,* lesdits au moins deux polypeptides consistant en la séquence de MTBN4, comme décrit dans la Figure 1, et
au moins un autre polypeptide du complexe de *M. tuberculosis,* qui n'est pas codé par le génome des cellules de la souche du BCG de *M. bovis.*

12. Vecteur comprenant ;

(a) la molécule d'ADN codant pour le polypeptide MTBN4, comme décrit dans la Figure 1 ; et
(b) les séquences de régulation de transcription et de traduction liées à ladite séquence d'ADN, lesdites séquences de régulation permettant l'expression du polypeptide codé par ladite séquence d'ADN dans une cellule,

pour une utilisation en tant que diagnostic.

13. Utilisation d'un polypeptide comprenant la séquence MTBN4, comme décrit dans la Figure 1, une composition telle que définie dans la revendication 11, ou un vecteur tel que défini dans la revendication 12, pour la préparation d'un médicament pour diagnostiquer l'exposition d'un sujet à *M. tuberculosis.*

FIG. 1

MTBN1
MTAEPEVRTLREVVLDQLGTAESRAYKMWLPPLTNPVPLNELIARDRRQPLRFALGIMDE
PRRHLQDVWGVDVSGAGGNIGIGGAPQTGKSTLLQTMVMSAAATHSPRNVQFYCIDLGGG
GLIYLENLPHVGGVANRSEPDKVNRVVAEMQAVMRQRETTFKEHRVGSIGMYRQLRDDPS
QPVASDPYGDVFLIIDGWPGFVGEFPDLEGQVQDLAAQGLAFGVHVIISTPRWTELKSRV
RDYLGTKIEFRLGDVNETQIDRITREIPANRPGRAVSMEKHHLMIGVPRFDGVHSADNLV
EAITAGVTQIASQHTEQAPPVRVLPERIHLHELDPNPPGPESDYRTRWEIPIGLRETDLT
PAHCHMHTNPHLLIFGAAKSGKTTIAHAIARAICARNSPQQVRFMLADYRSGLLDAVPDT
HLLGAGAINRNSASLDEAVQALAVNLKKRLPPTDLTTAQLRSRSWWSGFDVVLLVDDWHM
IVGAAGGMPPMAPLAPLLPAAADIGLHIIVTCQMSQAYKATMDKFVGAAFGSGAPTMFLS
GEKQEFPSSEFKVKRRPPGQAFLVSPDGKEVIQAPYIEPPEEVFAAPPSAG*

MTBN2
MEKMSHDPIAADIGTQVSDNALHGVTAGSTALTSVTGLVPAGADEVSAQAATAFTSEGIQ
LLASNASAQDQLHRAGEAVQDVARTYSQIDDGAAGVFAE*

MTBN3
MLWHAMPPELNTARLMAGAGPAPMLAAAAGWQTLSAALDAQAVELTARLNSLGEAWTGGG
SDKALAAATPMVVWLQTASTQAKTRAMQATAQAAAYTQAMATTPSLPEIAANHITQAVLT
ATNFFGINTIPIALTEMDYFIRMWNQAALAMEVYQAETAVNTLFEKLEPMASILDPGASQ
STTNPIFGMPSPGSSTPVGQLPPAATQTLGQLGEMSGPMQQLTQPLQQVTSLFSQVGGTG
GGNPADEEAAQMGLLGTSPLSNHPLAGGSGPSAGAGLLRAESLPGAGGSLTRTPLMSQLI
EKPVAPSVMPAAAAGSSATGGAAPVGAGAMGQGAQSGGSTRPGLVAPAPLAQEREEDDED
DWDEEDDW*

MTBN4
MAEMKTDAATLAQEAGNFERISGDLKTQIDQVESTAGSLQGQWRGAAGTAAQAAVVRFQE
AANKQKQELDEISTNIRQAGVQYSRADEEQQQALSSQMGF*

MTBN5
MAADYDKLFRPHEGMEAPDDMAAQPFFDPSASFPPAPASANLPKPNGQTPPPTSDDLSER
FVSAPPPPPPPPPPPPPTPMPIAAGEPPSPEPAASKPPTPPMPIAGPEPAPPKPPTPPMP
IAGPEPAPPKPPTPPMPIAGPAPTPTESQLAPPRPPTPQTPTGAPQQPESPAPHVPSHGP
HQPRRTAPAPPWAKMPIGEPPPAPSRPSASPAEPPTRPAPQHSRRARRGHRYRTDTERNV
GKVATGPSIQARLRAEEASGAQLAPGTEPSPAPLGQPRSYLAPPTRPAPTEPPPSPSPQR
NSGRRAERRVHPDLAAQHAAAQPDSITAATTGGRRRKRAAPDLDATQKSLRPAAKGPKVK
KVKPQKPKATKPPKVVSQRGWRHWVHALTRINLGLSPDEKYELDLHARVRRNPRGSYQIA
VVGLKGGAGKTTLTAALGSTLAQVRADRILALDADPGAGNLADRVGRQSGATIADVLAEK
ELSHYNDIRAHTSVNAVNLEVLPAPEYSSAQRALSDADWHFIADPASRFYNLVLADCGAG
FFDPLTRGVLSTVSGVVVVASVSIDGAQQASVALDWLRNNGYQDLASRACVVINHIMPGE
PNVAVKDLVRHFEQQVQPGRVVVMPWDRHIAAGTEISLDLLDPIYKRKVLELAAALSDDF
ERAGRR*

FIG. 1 (continued)

MTBN6

LSAPAVAAGPTAAGATAARPATTRVTILTGRRMTDLVLPAAVPMETYIDDTVAVLSEVLE
DTPADVLGGFDFTAQGVWAFARPGSPPLKLDQSLDDAGVVDGSLLTLVSVSRTERYRPLV
EDVIDAIAVLDESPEFDRTALNRFVGAAIPLLTAPVIGMAMRAWWETGRSLWWPLAIGIL
GIAVLVGSFVANRFYQSGHLAECLLVTTYLLIATAAALAVPLPRGVNSLGAPQVAGAATA
VLFLTLMTRGGPRKRHELASFAVITAIAVIAAAAAFGYGYQDWVPAGGIAFGLFIVTNAA
KLTVAVARIALPPIPVPGETVDNEELLDPVATPEATSEETPTWQAIIASVPASAVRLTER
SKLAKQLLIGYVTSGTLILAAGAIAVVVRGHFFVHSLVVAGLITTVCGFRSRLYAERWCA
WALLAATVAIPTGLTAKLIIWYPHYAWLLLSVYLTVALVALVVVGSMAHVRRVSPVVKRT
LELIDGAMIAAIIPMLLWITGVYDTVRNIRF*

MTBN7

MAEPLAVDPTGLSAAAAKLAGLVFPQPPAPIAVSGTDSVVAAINETMPSIESLVSDGLPG
VKAALTRTASNMNAAADVYAKTDQSLGTSLSQYAFGSSGEGLAGVASVGGQPSQATQLLS
TPVSQVTTQLGETAAELAPRVVATVPQLVQLAPHAVQMSQNASPIAQTISQTAQQAAQSA
QGGSGPMPAQLASAEKPATEQAEPVHEVTNDDQGDQGDVQPAEVVAAARDEGAGASPGQQ
PGGGVPAQAMDTGAGARPAASPLAAPVDPSTPAPSTTTTL*

MTBN8

MSITRPTGSYARQMLDPGGWVEADEDTFYDRAQEYSQVLQRVTDVLDTCRQQKGHVFEGG
LWSGGAANAANGALGANINQLMTLQDYLATVITWHRHIAGLIEQAKSDIGNNVDGAQREI
DILENDPSLDADERHTAINSLVTATHGANVSLVAETAERVLESKNWKPPKNALEDLLQQK
SPPPPDVPTLVVPSPGTPGTPGTPITPGTPITPGTPITPIPGAPVTPITPTPGTPVTPVT
PGKPVTPVTPVKPGTPGEPTPITPVTPPVAPATPATPATPVTPAPAPHPQPAPAPAPSPG
PQPVTPATPGPSGPATPGTPGGEPAPHVKPAALAEQPGVPGQHAGGGTQSGPAHADESAA
SVTPAAASGVPGARAAAAAPSGTAVGAGARSSVGTAAASGAGSHAATGRAPVATSDKAAA
PSTRAASARTAPPARPPSTDHIDKPDRSESADDGTPVSMIPVSAARAARDAATAAASARQ
RGRGDALRLARRIAAALNASDNNAGDYGFFWITAVTTDGSIVVANSYGLAYIPDGMELPN
KVYLASADHAIPVDEIARCATYPVLAVQAWAAFHDMTLRAVIGTAEQLASSDPGVAKIVL
EPDDIPESGKMTGRSRLEVVDPSAAAQLADTTDQRLLDLLPPAPVDVNPPGDERHMLWFE
LMKPMTSTATGREAAHLRAFRAYAAHSQEIALHQAHTATDAAVQRVAVADWLYWQYVTGL
LDRALAAAC*

FIG. 2

mtbn1

```
1     atgactgctg aaccggaagt acggacgctg cgcgaggttg tgctggacca
51    gctcggcact gctgaatcgc gtgcgtacaa gatgtggctg ccgccgttga
101   ccaatccggt cccgctcaac gagctcatcg cccgtgatcg gcgacaaccc
151   ctgcgatttg ccctggggat catggatgaa ccgcgccgcc atctacagga
201   tgtgtggggc gtagacgttt ccggggccgg cggcaacatc ggtattgggg
251   gcgcacctca aaccgggaag tcgacgctac tgcagacgat ggtgatgtcg
301   gccgccgcca cacactcacc gcgcaacgtt cagttctatt gcatcgacct
351   aggtggcggc gggctgatct atctcgaaaa ccttccacac gtcggtgggg
401   tagccaatcg gtccgagccc gacaaggtca accgggtggt cgcagagatg
451   caagccgtca tgcggcaacg ggaaaccacc ttcaaggaac accgagtggg
501   ctcgatcggg atgtaccggc agctgcgtga cgatccaagt caacccgttg
551   cgtccgatcc atacggcgac gtctttctga tcatcgacgg atggcccggt
601   tttgtcggcg agttccccga ccttgagggg caggttcaag atctggccgc
651   ccaggggctg gcgttcggcg tccacgtcat catctccacg ccacgctgga
701   cagagctgaa gtcgcgtgtt cgcgactacc tcggcaccaa gatcgagttc
751   cggcttggtg acgtcaatga aacccagatc gaccggatta cccgcgagat
801   cccggcgaat cgtccgggtc gggcagtgtc gatggaaaag caccatctga
851   tgatcggcgt gcccaggttc gacggcgtgc acagcgccga taacctggtg
901   gaggcgatca ccgcgggggt gacgcagatc gcttcccagc acaccgaaca
951   ggcacctccg gtgcgggtcc tgccggagcg tatccacctg cacgaactcg
1001  acccgaaccc gccgggacca gagtccgact accgcactcg ctgggagatt
1051  ccgatcggct tgcgcgagac ggacctgacg ccggctcact gccacatgca
1101  cacgaacccg cacctactga tcttcggtgc ggccaaatcg ggcaagacga
1151  ccattgccca cgcgatcgcg cgcgccattt gtgcccgaaa cagtccccag
1201  caggtgcggt tcatgctcgc ggactaccgc tcgggcctgc tggacgcggt
1251  gccggacacc catctgctgg gcgccggcgc gatcaaccgc aacagcgcgt
1301  cgctagacga ggccgttcaa gcactggcgg tcaacctgaa gaagcggttg
1351  ccgccgaccg acctgacgac ggcgcagcta cgctcgcgtt cgtggtggag
1401  cggatttgac gtcgtgcttc tggtcgacga ttggcacatg atcgtgggtg
1451  ccgccggggg gatgccgccg atggcaccgc tggccccgtt attgccggcg
1501  gcggcagata tcgggttgca catcattgtc acctgtcaga tgagccaggc
1551  ttacaaggca accatggaca agttcgtcgg cgccgcattc gggtcgggcg
1601  ctccgacaat gttcctttcg ggcgagaagc aggaattccc atccagtgag
1651  ttcaaggtca agcggcgccc ccctggccag gcatttctcg tctcgccaga
1701  cggcaaagag gtcatccagg ccccctacat cgagcctcca gaagaagtgt
1751  tcgcagcacc cccaagcgcc ggttaa
```

mtbn2

```
1     atggaaaaaa tgtcacatga tccgatcgct gccgacattg gcacgcaagt
51    gagcgacaac gctctgcacg gcgtgacggc cggctcgacg gcgctgacgt
101   cggtgaccgg gctggttccc gcgggggccg atgaggtctc cgcccaagcg
151   gcgacggcgt tcacatcgga gggcatccaa ttgctggctt ccaatgcatc
201   ggcccaagac cagctccacc gtgcgggcga agcggtccag gacgtcgccc
251   gcacctattc gcaaatcgac gacggcgccg ccggcgtctt cgcctaatag
```

mtbn3

```
1     atgctgtggc acgcaatgcc accggagcta aataccgcac ggctgatggc
51    cggcgcgggt ccggctccaa tgcttgcggc ggccgcggga tggcagacgc
101   tttcggcggc tctggacgct caggccgtcg agttgaccgc gcgcctgaac
```

FIG. 2 (continued)

```
151  tctctgggag aagcctggac tggaggtggc agcgacaagg cgcttgcggc
201  tgcaacgccg atggtggtct ggctacaaac cgcgtcaaca caggccaaga
251  cccgtgcgat gcaggcgacg gcgcaagccg cggcatacac ccaggccatg
301  gccacgacgc cgtcgctgcc ggagatcgcc gccaaccaca tcacccaggc
351  cgtccttacg gccaccaact tcttcggtat caacacgatc ccgatcgcgt
401  tgaccgagat ggattatttc atccgtatgt ggaaccaggc agccctggca
451  atggaggtct accaggccga gaccgcggtt aacacgcttt tcgagaagct
501  cgagccgatg gcgtcgatcc ttgatcccgg cgcgagccag agcacgacga
551  acccgatctt cggaatgccc tcccctggca gctcaacacc ggttggccag
601  ttgccgccgg cggctaccca gaccctcggc caactgggtg agatgagcgg
651  cccgatgcag cagctgaccc agccgctgca gcaggtgacg tcgttgttca
701  gccaggtggg cggcaccggc ggcggcaacc cagccgacga ggaagccgcg
751  cagatgggcc tgctcggcac cagtccgctg tcgaaccatc cgctggctgg
801  tggatcaggc cccagcgcgg gcgcgggcct gctgcgcgcg gagtcgctac
851  ctggcgcagg tgggtcgttg acccgcacgc cgctgatgtc tcagctgatc
901  gaaaagccgg ttgccccctc ggtgatgccg gcggctgctg ccggatcgtc
951  ggcgacgggt ggcgccgctc cggtgggtgc gggagcgatg ggccagggtg
1001 cgcaatccgg cggctccacc aggccgggtc tggtcgcgcc ggcaccgctc
1051 gcgcaggagc gtgaagaaga cgacgaggac gactgggacg aagaggacga
1101 ctggtga
```

mtbn4

```
1    atggcagaga tgaagaccga tgccgctacc ctcgcgcagg aggcaggtaa
51   tttcgagcgg atctccggcg acctgaaaac ccagatcgac caggtggagt
101  cgacggcagg ttcgttgcag ggccagtggc gcggcgcggc ggggacggcc
151  gcccaggccg cggtggtgcg cttccaagaa gcagccaata agcagaagca
201  ggaactcgac gagatctcga cgaatattcg tcaggccggc gtccaatact
251  cgagggccga cgaggagcag cagcaggcgc tgtcctcgca aatgggcttc
301  tga
```

mtbn5

```
1    atggcggccg actacgacaa gctcttccgg ccgcacgaag gtatggaagc
51   tccggacgat atggcagcgc agccgttctt cgaccccagt gcttcgtttc
101  cgccggcgcc cgcatcggca aacctaccga agcccaacgg ccagactccg
151  cccccgacgt ccgacgacct gtcggagcgg ttcgtgtcgg ccccgccgcc
201  gccacccccа cccccacctc cgcctccgcc aactccgatg ccgatcgccg
251  caggagagcc gccctcgccg gaaccggccg catctaaacc acccacaccc
301  cccatgccca tcgccggacc cgaaccggcc ccacccaaac cacccacacc
351  ccccatgccc atcgccggac ccgaaccggc cccacccaaa ccacccacac
401  ctccgatgcc catcgccgga cctgcaccca ccccaaccga atcccagttg
451  gcgcccccca gaccaccgac accacaaacg ccaaccggag cgccgcagca
501  accggaatca ccggcgcccc acgtaccctc gcacgggcca catcaacccc
551  ggcgcaccgc accagcaccg ccctgggcaa agatgccaat cggcgaaccc
601  ccgcccgctc cgtccagacc gtctgcgtcc ccggccgaac caccgacccg
651  gcctgccccc caacactccc gacgtgcgcg ccggggtcac cgctatcgca
701  cagacaccga acgaaacgtc gggaaggtag caactggtcc atccatccag
751  gcgcggctgc gggcagagga agcatccggc gcgcagctcg ccccggaac
801  ggagccctcg ccagcgccgt tgggccaacc gagatcgtat ctggctccgc
851  ccacccgccc cgcgccgaca gaacctcccc ccagcccctc gccgcagcgc
901  aactccggtc ggcgtgccga gcgacgcgtc caccccgatt tagccgccca
```

FIG. 2 (continued)

```
951  acatgccgcg gcgcaacctg attcaattac ggccgcaacc actggcggtc
1001 gtcgccgcaa gcgtgcagcg ccggatctcg acgcgacaca gaaatcctta
1051 aggccggcgg ccaaggggcc gaaggtgaag aaggtgaagc cccagaaacc
1101 gaaggccacg aagccgccca aagtggtgtc gcagcgcggc tggcgacatt
1151 gggtgcatgc gttgacgcga atcaacctgg gcctgtcacc cgacgagaag
1201 tacgagctgg acctgcacgc tcgagtccgc cgcaatcccc gcgggtcgta
1251 tcagatcgcc gtcgtcggtc tcaaaggtgg ggctggcaaa accacgctga
1301 cagcagcgtt ggggtcgacg ttggctcagg tgcgggccga ccggatcctg
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1401 acaatcgggc gcgaccatcg ctgatgtgct tgcagaaaaa gagctgtcgc
1451 actacaacga catccgcgca cacactagcg tcaatgcggt caatctggaa
1501 gtgctgccgg caccggaata cagctcggcg cagcgcgcgc tcagcgacgc
1551 cgactggcat ttcatcgccg atcctgcgtc gaggttttac aacctcgtct
1601 tggctgattg tggggccggc ttcttcgacc cgctgacccg cggcgtgctg
1651 tccacggtgt ccggtgtcgt ggtcgtggca agtgtctcaa tcgacggcgc
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1801 cccaatgtcg cagttaaaga cctggtgcgg catttcgaac agcaagttca
1851 acccggccgg gtcgtggtca tgccgtggga caggcacatt gcggccggaa
1901 ccgagatttc actcgacttg ctcgacccta tctacaagcg caaggtcctc
1951 gaattggccg cagcgctatc cgacgatttc gagagggctg gacgtcgttg
2001  a
```

mtbn6

```
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51   tgcgcggcct gccaccaccc gggtgacgat cctgaccggc agacggatga
101  ccgatttggt actgccagcg gcggtgccga tggaaactta tattgacgac
151  accgtcgcgg tgctttccga ggtgttggaa gacacgccgg ctgatgtact
201  cggcggcttc gactttaccg cgcaaggcgt gtgggcgttc gctcgtcccg
251  gatcgccgcc gctgaagctc gaccagtcac tcgatgacgc cggggtggtc
301  gacgggtcac tgctgactct ggtgtcagtc agtcgcaccg agcgctaccg
351  accgttggtc gaggatgtca tcgacgcgat cgccgtgctt gacgagtcac
401  ctgagttcga ccgcacggca ttgaatcgct ttgtgggggc ggcgatcccg
451  cttttgaccg cgcccgtcat cgggatggcg atgcgggcgt ggtgggaaac
501  tgggcgtagc ttgtggtggc cgttggcgat tggcatcctg gggatcgctg
551  tgctggtagg cagcttcgtc gcgaacaggt tctaccagag cggccacctg
601  gccgagtgcc tactggtcac gacgtatctg ctgatcgcaa ccgccgcagc
651  gctggccgtg ccgttgccgc gcggggtcaa ctcgttgggg gcgccacaag
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901  aagctgaccg tcgcggtcgc gcggatcgcg ctgccgccga ttccggtacc
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1201 ggtttgatca cgaccgtctg cggatttcgc tcgcggcttt acgccgagcg
1251 ctggtgtgcg tgggcgttgc tggcggcgac ggtcgcgatt ccgacgggtc
1301 tgacggccaa actcatcatc tggtacccgc actatgcctg gctgttgttg
```

FIG. 2 (continued)

```
1351 agcgtctacc tcacggtagc cctggttgcg ctcgtggtgg tcgggtcgat
1401 ggctcacgtc cggcgcgttt caccggtcgt aaaacgaact ctggaattga
1451 tcgacggcgc catgatcgct gccatcattc ccatgctgct gtggatcacc
1501 ggggtgtacg acacggtccg caatatccgg ttctga
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mtbn7

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201  aacagcatcc aacatgaacg cggcggcgga cgtctatgcg aagaccgatc
251  agtcactggg aaccagtttg agccagtatg cattcggctc gtcgggcgaa
301  ggcctggctg gcgtcgcctc ggtcggtggt cagccaagtc aggctaccca
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401  ccgctgagct ggcaccccgt gttgttgcga cggtgccgca actcgttcag
451  ctggctccgc acgccgttca gatgtcgcaa aacgcatccc ccatcgctca
501  gacgatcagt caaaccgccc aacaggccgc ccagagcgcg cagggcggca
551  gcggcccaat gcccgcacag cttgccagcg ctgaaaaacc ggccaccgag
601  caagcggagc cggtccacga agtgacaaac gacgatcagg gcgaccaggg
651  cgacgtgcag ccggccgagg tcgttgccgc ggcacgtgac gaaggcgccg
701  gcgcatcacc gggccagcag cccggcgggg gcgttcccgc gcaagccatg
751  gataccggag ccggtgcccg cccagcggcg agtccgctgg cggcccccgt
801  cgatccgtcg actccggcac cctcaacaac cacaacgttg tag
```

mtbn8

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1    atgagtatta ccaggccgac gggcagctat gccagacaga tgctggatcc
51   gggcggctgg gtggaagccg atgaagacac tttctatgac cgggcccagg
101  aatatagcca ggttttgcaa agggtcaccg atgtattgga cacctgccgc
151  cagcagaaag gccacgtctt cgaaggcggc ctatggtccg gcggcgccgc
201  caatgctgcc aacggcgccc tgggtgcaaa catcaatcaa ttgatgacgc
251  tgcaggatta tctcgccacg gtgattacct ggcacaggca tattgccggg
301  ttgattgagc aagctaaatc cgatatcggc aataatgtgg atggcgctca
351  acgggagatc gatatcctgg agaatgaccc tagcctggat gctgatgagc
401  gccataccgc catcaattca ttggtcacgg cgacgcatgg ggccaatgtc
451  agtctggtcg ccgagaccgc tgagcgggtg ctggaatcca agaattggaa
501  acctccgaag aacgcactcg aggatttgct tcagcagaag tcgccgccac
551  ccccagacgt gcctaccctg gtcgtgccat ccccgggcac accgggcaca
601  ccgggaaccc cgatcacccc gggaaccccg atcaccccgg gaaccccaat
651  cacacccatc ccgggagcgc cggtaactcc gatcacacca acgcccggca
701  ctcccgtcac gccggtgacc ccgggcaagc cggtcacccc ggtgaccccg
751  gtcaaaccgg gcacaccagg cgagccaacc ccgatcacgc cggtcacccc
801  cccggtcgcc ccggccacac cggcaacccc ggccacgccc gttaccccag
851  ctcccgctcc acacccgcag ccggctccgg caccggcgcc atcgcctggg
901  ccccagccgg ttacaccggc cactcccggt ccgtctggtc cagcaacacc
951  gggcacccca gggggcgagc cggcgccgca cgtcaaaccc gcggcgttgg
1001 cggagcaacc tggtgtgccg ggccagcatg cgggcggggg gacgcagtcg
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1101 gtccggtgtc ccgggcgcac gggcggcggc cgccgcgccg agcggtaccg
1151 ccgtgggagc gggcgcgcgt tcgagcgtgg gtacggccgc ggcctcgggc
1201 gcggggtcgc atgctgccac tgggcgggcg ccggtggcta cctcggacaa
```

FIG. 2 (continued)

```
1251 ggcggcggca ccgagcacgc gggcggcctc ggcgcggacg gcacctcctg
1301 cccgcccgcc gtcgaccgat cacatcgaca aacccgatcg cagcgagtct
1351 gcagatgacg gtacgccggt gtcgatgatc ccggtgtcgg cggctcgggc
1401 ggcacgcgac gccgccactg cagctgccag cgcccgccag cgtggccgcg
1451 gtgatgcgct gcggttggcg cgacgcatcg cggcggcgct caacgcgtcc
1501 gacaacaacg cgggcgacta cgggttcttc tggatcaccg cggtgaccac
1551 cgacggttcc atcgtcgtgg ccaacagcta tgggctggcc tacatacccg
1601 acgggatgga attgccgaat aaggtgtact tggccagcgc ggatcacgca
1651 atcccggttg acgaaattgc acgctgtgcc acctacccgg ttttggccgt
1701 gcaagcctgg gcggctttcc acgacatgac gctgcgggcg gtgatcggta
1751 ccgcggagca gttggccagt tcggatcccg gtgtggccaa gattgtgctg
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1901 agcgtttgct cgacttgttg ccgccggcgc cggtggatgt caatccaccg
1951 ggcgatgagc ggcacatgct gtggttcgag ctgatgaagc ccatgaccag
2001 caccgctacc ggccgcgagg ccgctcatct gcgggcgttc cgggcctacg
2051 ctgcccactc acaggagatt gccctgcacc aagcgcacac tgcgactgac
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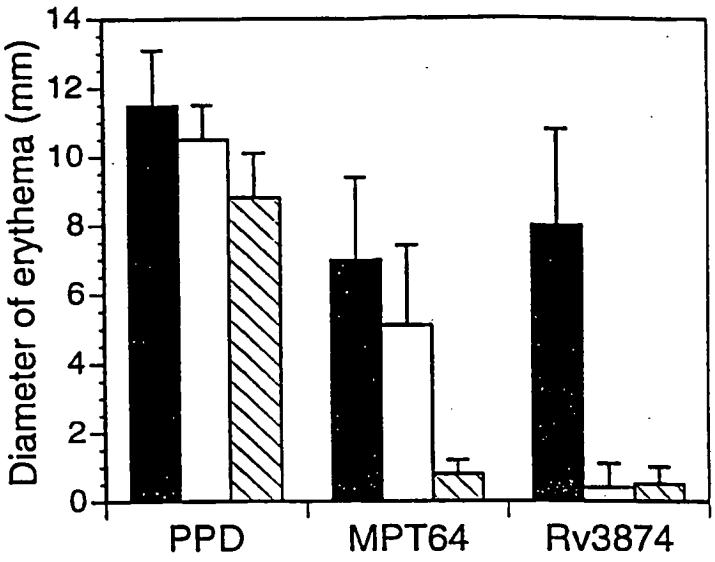

Fig.3

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 796792 A **[0031] [0033]**

Non-patent literature cited in the description

- **COLE et al.** *Nature,* 1998, vol. 393, 537-544 **[0029]**
- **MAHAIRAS et al.** *J. Bacteriol.,* 1996, vol. 178, 1274-1282 **[0030]**

专利名称(译)	由结核分枝杆菌而不是BCG表达的蛋白质及其作为诊断试剂和疫苗的用途		
公开(公告)号	EP1214088B1	公开(公告)日	2009-04-22
申请号	EP2000928851	申请日	2000-05-04
[标]申请(专利权)人(译)	公共卫生RES INST纽约市的		
当前申请(专利权)人(译)	罗格斯新泽西州立大学		
[标]发明人	GENNARO MARIA L		
发明人	GENNARO, MARIA, L.		
IPC分类号	A61K39/00 A61K39/02 A61K39/04 A61K38/00 C07H21/02 C07H21/04 C07K1/00 C12P21/06 C12N1/00 C12N1/12 G01N33/53 A61K31/711 A61K48/00 A61P31/04 A61P31/06 C07K14/35 C12N1/15 C12N1/19 C12N1/21 C12N5/10 C12N15/09 C12Q1/02		
CPC分类号	A61K49/0006 A61K38/00 A61K39/00 A61K39/04 A61K2039/53 C07K14/35 G01N33/5091 G01N33/5695 G01N2333/35 G01N2333/57 G01N2800/26 Y10S435/863		
优先权	60/132505 1999-05-04 US		
其他公开文献	EP1214088A4 EP1214088A1		
外部链接	Espacenet		

摘要(译)

本发明提供了由存在于结核分枝杆菌基因组中但不存在于BCG基因组中的开放阅读框编码的多肽以及使用这些多肽的诊断和预防方法。

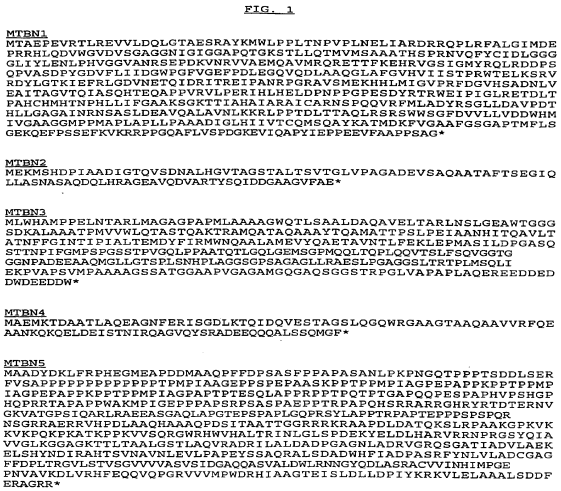
FIG. 1

MTBN1
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PRRHLQDVWGVDVSGAGGNIGIGGAPQTGKSTLLQTMVMSAAATHSPRNVQFYCIDLGGG
GLIYLENLPHVGGVANRSEPDKVNRVVAEMQAVMRQRETTFKEHRVGSIGMYRQLRDDPS
QPVASDPYGDVFLIIDGWPGFVGEFPDLEGQVQDLAAQGLAFGVHVIISTPRWTELKSRV
RDYLGTKIEFRLGDVNETQIDRITREIPANRPGRAVSMEKHHLMIGVPRFDGVHSADNLV
EAITAGVTQIASQHTEQAPPVRVLPERIHLHELDPNPPGPESDYRTRWEIPIGLRETDLT
PAHCHMHTNPHLLIFGAAKSGKTTIAHAIARAICARNSPQQVRFMLADYRSGLLDAVPDT
HLLGAGAINRNSASLDEAVQALAVNLKKRLPPTDLTTAQLRSRSWWSGFDVVLLVDDWHM
IVGAAGGMPPMAPLAPLLPAAADIGLHIIVTCQMSQAYKATMDKFVGAAPGSGAPTMFLS
GEKQEFPSSEFKVKRRPPGQAFLVSPDGKEVIQAPYIEPPEEVFAAPPSAG*

MTBN2
MEKMSHDPIAADIGTQVSDNALHGVTAGSTALTSVTGLVPAGADEVSAQAATAFTSEGIQ
LLASNASAQDQLHRAGEAVQDVARTYSQIDDGAAGVFAE*

MTBN3
MLWHAMPPELNTARLMAGAGPAPMLAAAAGWQTLSAALDAQAVELTARLNSLGEAWTGGG
SDKALAAATPMVVWLQTAGTQAKTRAMQATAQAAAYTQAMATTPSLPEIAANHITQAVLT
ATNFFGINTIPIALTEMDYFIRMWNQAALAMEVYQAETAVNTLFEKLEPMASILDPGASQ
STTNPIFGMPSPGSSTPVGQLPPAATQTLGQLGEMSGPFMQQLTQPLQQVTSLFSQVGGTG
GGNPADEEAAQMGLLGTSPLSNHPIAGGSGPSAGAGLLRAESLPGAGGSLTKTPLMSQLI
EKPVAPSVMPAAAAGSSATGGAAPVGAGAMGQGAQSGGSTRPGLVAPAPLAQEREEDDED
DWDEEDDW*

MTBN4
MAEMKTDAATLAQEAGNFERISGDLKTQIDQVESTAGSLQGQWRGAAGTAAQAAVVRFQE
AANKQKQELDEISTNIRQAGVQYSRADEEQQQALSSQMGF*

MTBN5
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IAGPEPAPPKPPTPPMPIAGPAPTPTESQLAPPRPPTPQTPTGAPQQPESPAPHVPSHGP
HQPRRTAPAPPWAKMPIGEPPPAPSRPSASPAEPPTPRPAPQHSRRARRGHRYRTDTERNV
GKVATGPSIQARLRAEEASGAQLAPGTEPSPAPLGQPKSYLAPPTKPAPTEPPPSPSPQR
NSGRRAERRVHPDLAAQHAAAQPDSITAATTGGRRRKRAAPDLDATQKSLRPAAKGPKVK
KVKPQKPKATKPPKVVSQRGWRHWVHALTRINLGLSPDEKYELDLHARVRRNPRGSYQIA
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ELSHYNDIRAHTSVNAVNLEVLPAPEYSSAQRALSDADWHFIADPASRFYNLVLADCGAG
FFDPLTRGVLSTVSGVVVVASVSIDGAQQASVALDWLRNNGYQDLASRACVVINHIMPGE
PNVAVKDLVRHFEQQVQPGRVVVMPWDRHIAAGTEISLDLLDPIYKRKVLELAAALSDDF
ERAGRR*