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Brown et al.(10) **Pub. No.: US 2011/0206712 A1**(43) **Pub. Date: Aug. 25, 2011**(54) **TUBERCULOSIS RV2386C PROTEIN,
COMPOSITIONS AND USES THEREOF****Publication Classification**(76) Inventors: **James Brown**, Collegeville, PA
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A61P 31/06 (2006.01)
A61P 37/04 (2006.01)(21) Appl. No.: **13/055,787**(52) **U.S. Cl.** **424/190.1**; 424/248.1; 530/350;
536/23.4(22) PCT Filed: **Jul. 24, 2009**(86) PCT No.: **PCT/EP2009/059585**(57) **ABSTRACT**§ 371 (c)(1),
(2), (4) Date: **Jan. 25, 2011****Related U.S. Application Data**(60) Provisional application No. 61/083,699, filed on Jul.
25, 2008.

The present invention is directed to a polypeptide which comprises: (i) an Rv2386c protein sequence; (ii) a variant of an Rv2386c protein sequence; or (iii) an immunogenic fragment of an Rv2386c protein sequence. In other aspects the invention is directed to associated polynucleotides, fusion proteins and methods for the treatment or prevention of tuberculosis.

Figure 1

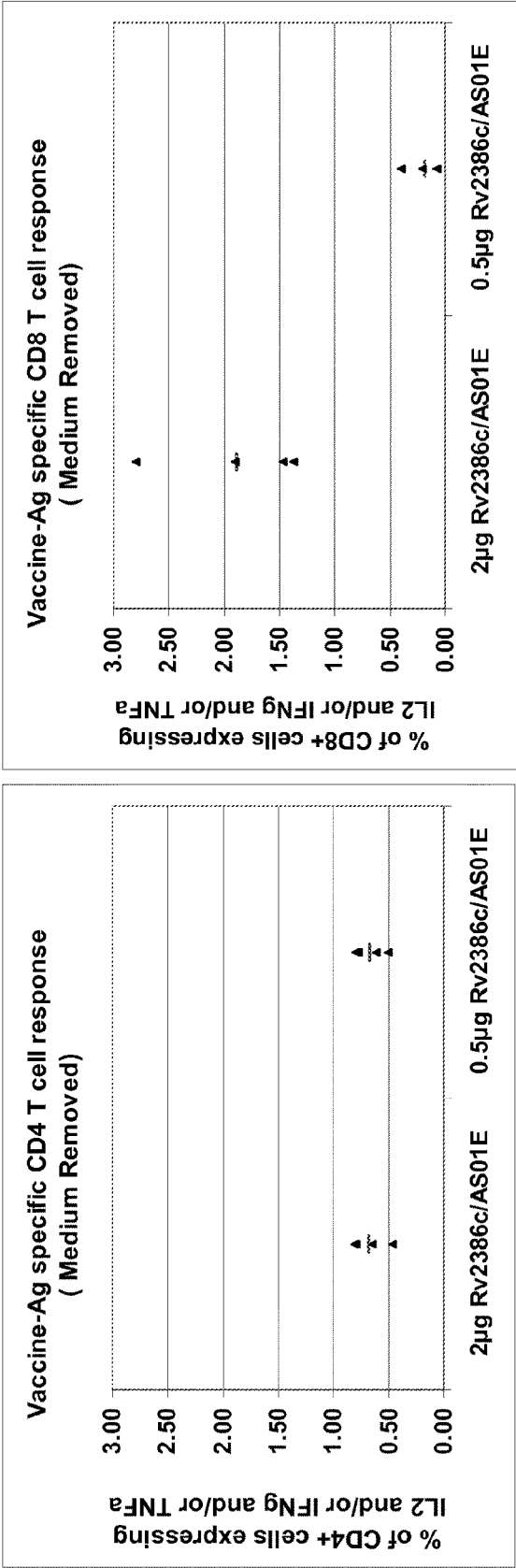


Figure 2

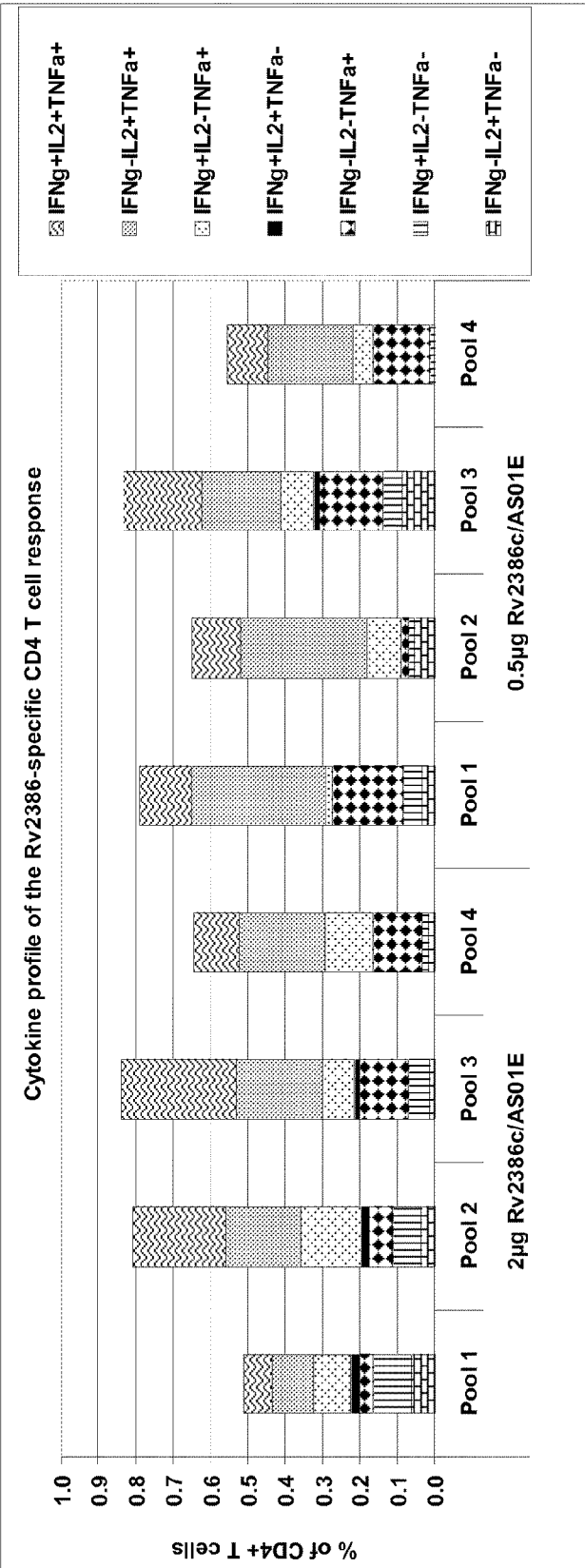


Figure 3

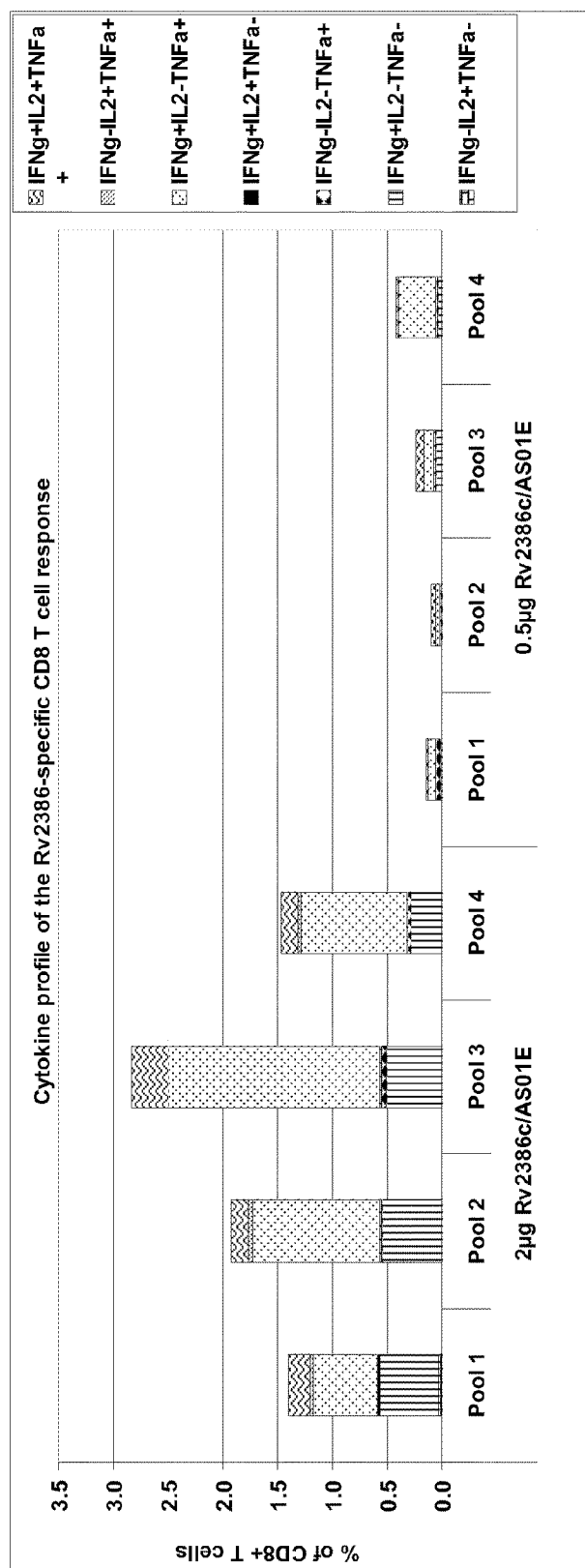


Figure 4

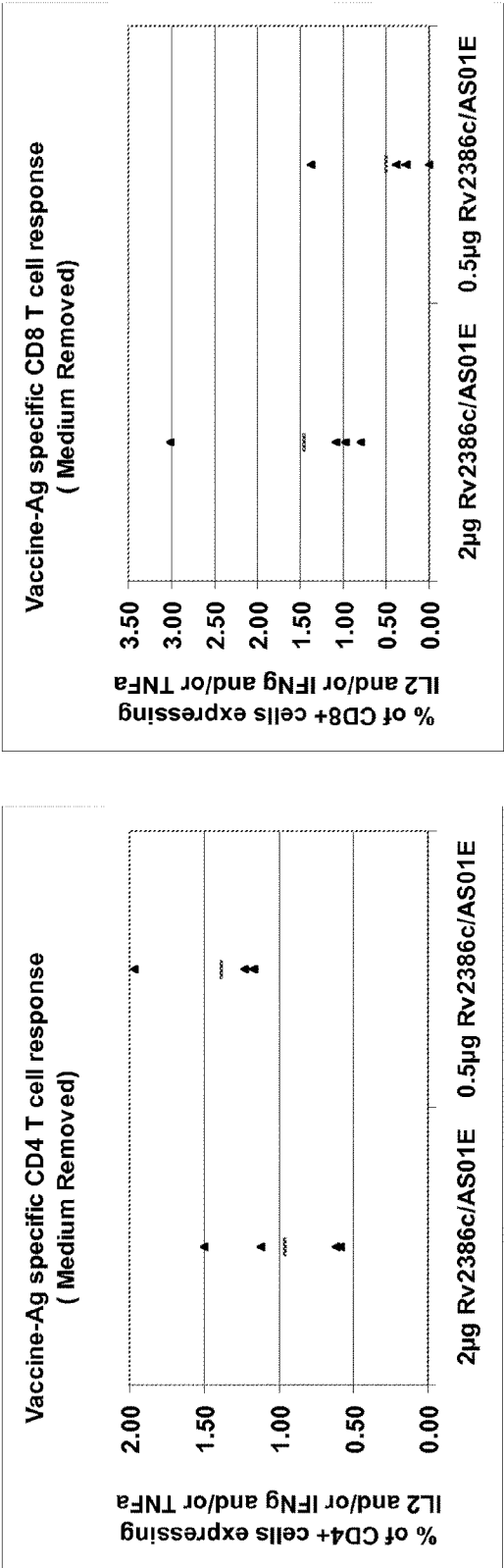


Figure 5

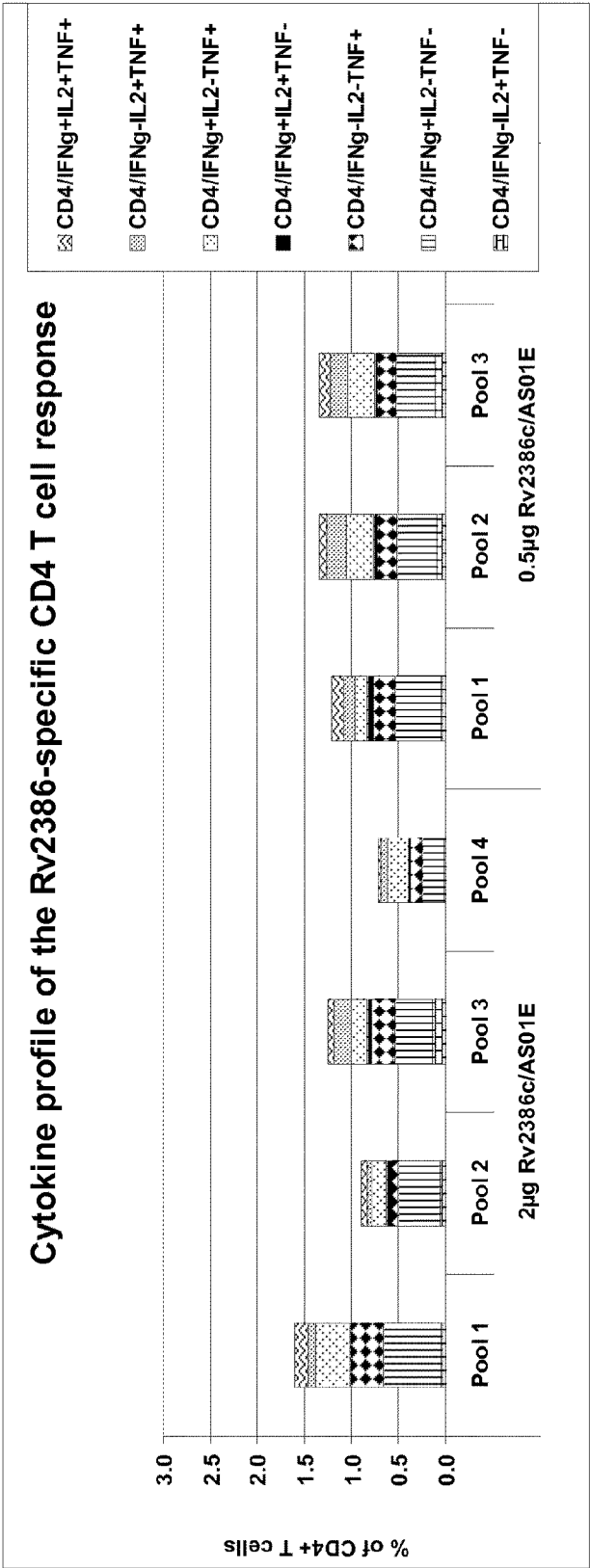


Figure 6

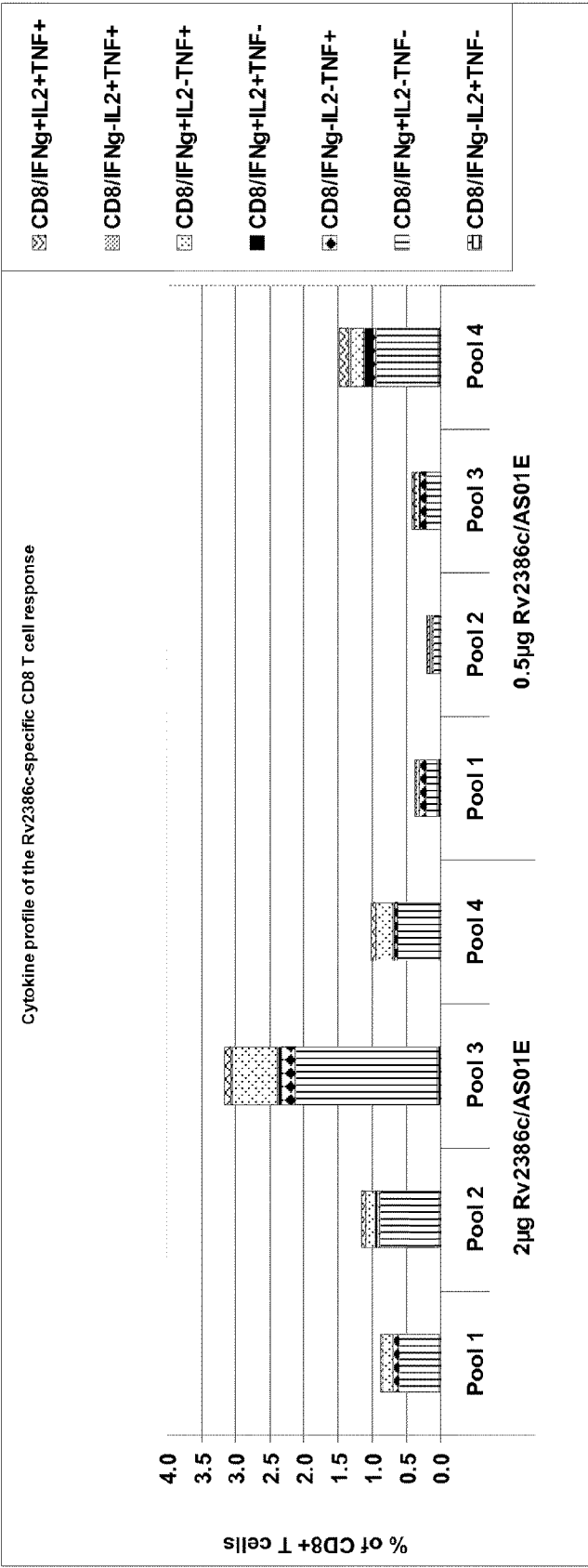


Figure 7

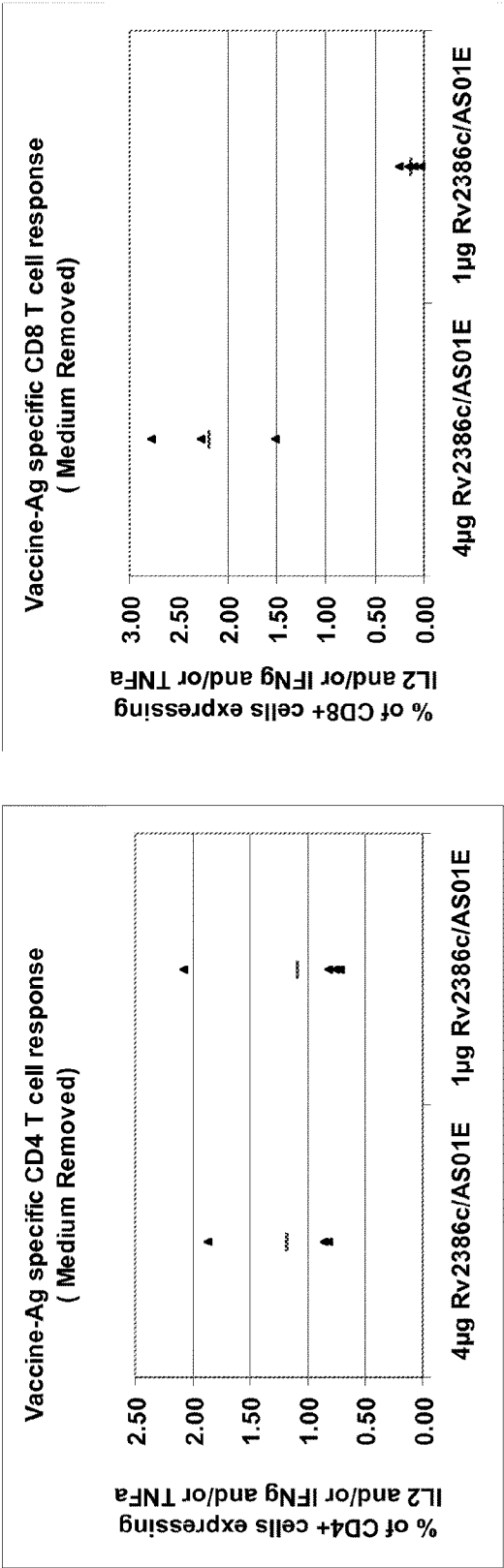


Figure 8

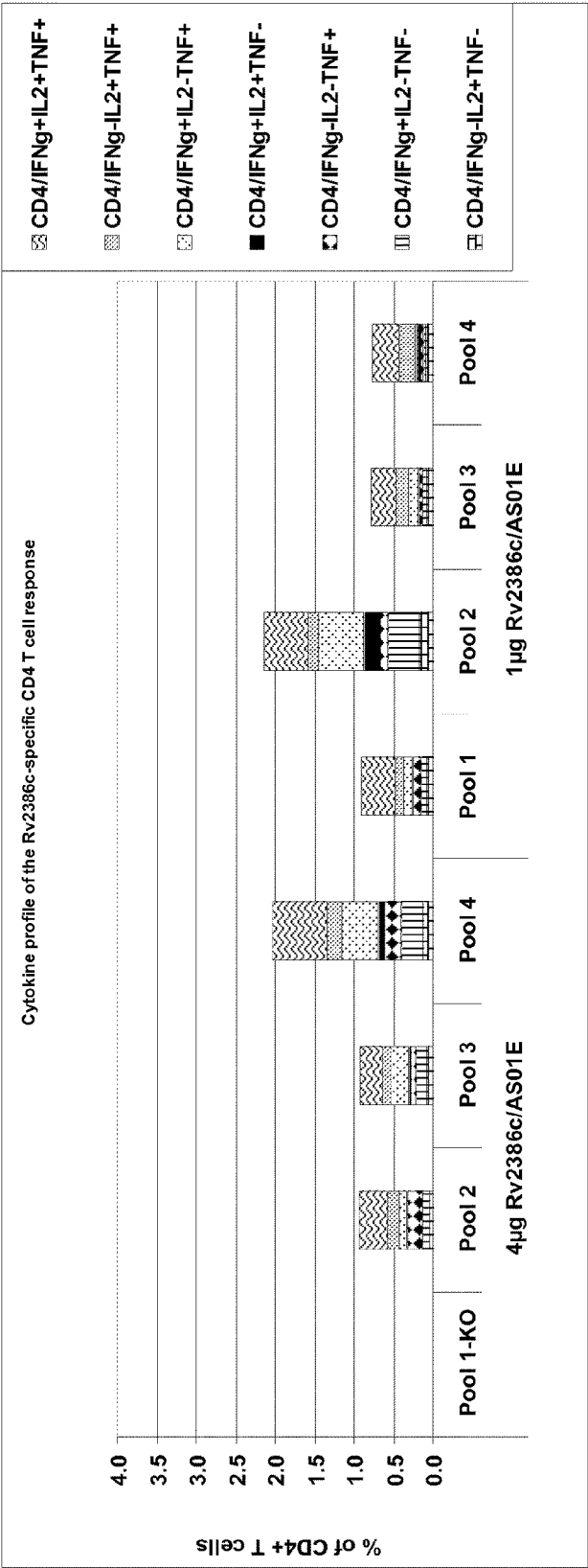
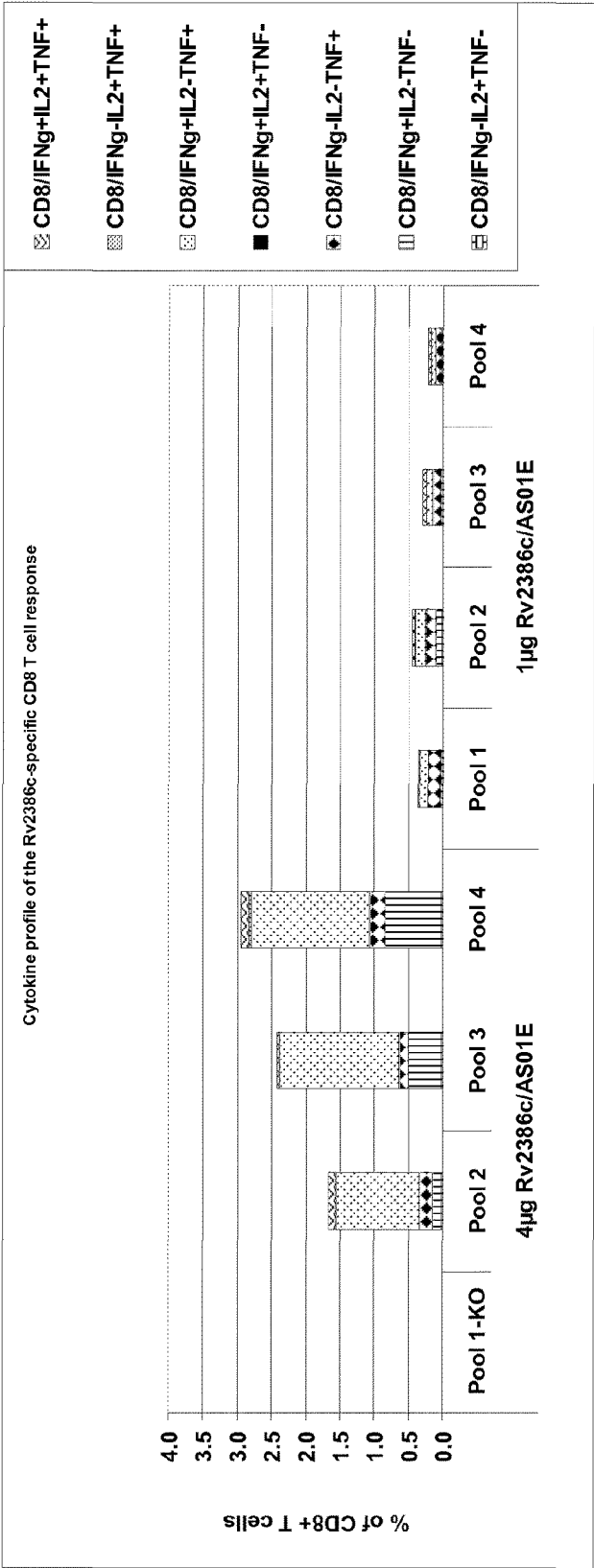


Figure 9



TUBERCULOSIS RV2386C PROTEIN, COMPOSITIONS AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to polypeptides and polynucleotides for use in the treatment or prevention of tuberculosis, in particular for use in the treatment or prevention of latent tuberculosis and in the prevention or delay of reactivation of tuberculosis (and also to related methods). The present invention further relates to pharmaceutical and immunogenic compositions comprising said polypeptides and polynucleotides, and to methods for the diagnosis of tuberculosis (in particular latent tuberculosis).

BACKGROUND OF THE INVENTION

[0002] Tuberculosis (TB) is a chronic infectious disease caused by infection with *Mycobacterium tuberculosis* and other *Mycobacterium* species. It is a major disease in developing countries, as well as an increasing problem in developed areas of the world. More than 2 billion people are believed to be infected with TB bacilli, with about 9.2 million new cases of TB and 1.7 million deaths each year. 10% of those infected with TB bacilli will develop active TB, each person with active TB infecting an average of 10 to 15 others per year. While annual incidence rates have peaked globally, the number of deaths and cases is still rising due to population growth (World Health Organisation *Tuberculosis Facts* 2008).

[0003] *Mycobacterium tuberculosis* infects individuals through the respiratory route. Alveolar macrophages engulf the bacterium, but it is able to survive and proliferate by inhibiting phagosome fusion with acidic lysosomes. A complex immune response involving CD4+ and CD8+ T cells ensues, ultimately resulting in the formation of a granuloma. Central to the success of *Mycobacterium tuberculosis* as a pathogen is the fact that the isolated, but not eradicated, bacterium may persist for long periods, leaving an individual vulnerable to the later development of active TB.

[0004] Fewer than 5% of infected individuals develop active TB in the first years after infection. The granuloma can persist for decades and is believed to contain live *Mycobacterium tuberculosis* in a state of dormancy, deprived of oxygen and nutrients. However, recently it has been suggested that the majority of the bacteria in the dormancy state are located in non-macrophage cell types spread throughout the body (Locht et al, *Expert Opin. Biol. Ther.* 2007 7(11):1665-1677). The development of active TB occurs when the balance between the host's natural immunity and the pathogen changes, for example as a result of an immunosuppressive event (Anderson P *Trends in Microbiology* 2007 15(1):7-13; Ehlers S *Infection* 2009 37(2):87-95).

[0005] A dynamic hypothesis describing the balance between latent TB and active TB has also been proposed (Cardana P-J *Inflammation & Allergy—Drug Targets* 2006 6:27-39; Cardana P-J *Infection* 2009 37(2):80-86).

[0006] Although an infection may be asymptomatic for a considerable period of time, the active disease is most commonly manifested as an acute inflammation of the lungs, resulting in tiredness, weight loss, fever and a persistent cough. If untreated, serious complications and death typically result.

[0007] Tuberculosis can generally be controlled using extended antibiotic therapy, although such treatment is not

sufficient to prevent the spread of the disease. Infected individuals may be asymptomatic, but contagious, for some time. In addition, although compliance with the treatment regimen is critical, patient behaviour is difficult to monitor. Some patients do not complete the course of treatment, which can lead to ineffective treatment and the development of drug resistance.

[0008] Multidrug-resistant TB (MDR-TB) is a form which fails to respond to first line medications. 5% of all TB cases are MDR-TB, with an estimated 490,000 new MDR-TB cases occurring each year. Extensively drug-resistant TB (XDR-TB) occurs when resistance to second line medications develops on top of MDR-TB. It is estimated that 40,000 new cases of the virtually untreatable XDR-TB arise annually (World Health Organisation *Tuberculosis Facts* 2008).

[0009] Even if a full course of antibiotic treatment is completed, infection with *M. tuberculosis* may not be eradicated from the infected individual and may remain as a latent infection that can be reactivated.

[0010] In order to control the spread of tuberculosis, effective vaccination and accurate early diagnosis of the disease are of utmost importance.

[0011] Diagnosis of latent TB infection is commonly achieved using the tuberculin skin test, which involves intradermal exposure to tuberculin protein-purified derivative (PPD). Antigen-specific T cell responses result in measurable induration at the injection site by 48-72 hours after injection, which indicates exposure to mycobacterial antigens. Sensitivity and specificity have, however, been a problem with this test, and individuals vaccinated with BCG cannot always be easily distinguished from infected individuals (this is particularly important in light of the fact that BCG does not protect against latent infection). In general, individuals who have received BCG but are not infected by *M. tuberculosis* show a PPD reaction below 10 mm in diameter whereas people who have a PPD reaction above 10 mm in diameter are considered to have been infected by *M. tuberculosis*. However, this rule is not applicable to individuals with immunosuppression due to HIV infection, which may result in a PPD reaction below 10 mm in diameter; or in endemic countries, where people infected by non-tuberculosis mycobacteria can show a PPD reaction above 10 mm in diameter.

[0012] Progress over recent years has seen the development of in vitro T cell based assays, based on interferon-gamma release and using antigens which are more specific to *M. tuberculosis* than PPD, namely ESAT-6 and CFP-10. These high specificity tests appear to be at least as sensitive as the tuberculin skin test and also demonstrate less cross-reactivity due to BCG vaccination. See Pai M et al *Expert Rev. Mol. Diagn.* 2006 6(3):413-422 for a recent review of latent TB diagnosis. However, since ESAT-6/CFP-10 are early stage antigens, assays based on ESAT-6/CFP-10 may only perform optimally in recently infected people. Consequently, the identification of new antigens specifically associated with latent tuberculosis may aid the development of more sensitive assays that could detect longer-term latent infections.

[0013] There remains a need for effective strategies for the treatment and prevention of tuberculosis, in particular the treatment and prevention of latent TB and the prevention of reactivation of TB.

BRIEF SUMMARY OF THE INVENTION

[0014] The present invention relates generally to the identification of RV2386c as a TB antigen (in particular an antigen

associated with latent TB) and to related methods and uses in the prevention and treatment of TB, especially the prevention and treatment of latent TB and the prevention or delay of TB reactivation.

[0015] The present invention provides an isolated polypeptide which comprises:

- [0016] (i) an Rv2386c protein sequence;
- [0017] (ii) a variant of an Rv2386c protein sequence; or
- [0018] (iii) an immunogenic fragment of an Rv2386c protein sequence.

[0019] The present invention also provides a polypeptide which comprises:

- [0020] (i) an Rv2386c protein sequence;
- [0021] (ii) a variant of an Rv2386c protein sequence; or
- [0022] (iii) an immunogenic fragment of an Rv2386c protein sequence; for use as a medicament.

[0023] A further aspect of the invention relates to a method for the treatment or prevention of TB comprising the administration of a safe and effective amount of a polypeptide comprising:

- [0024] (i) an Rv2386c protein sequence;
- [0025] (ii) a variant of an Rv2386c protein sequence; or
- [0026] (iii) an immunogenic fragment of an Rv2386c protein sequence;

to a subject in need thereof, wherein said polypeptide induces an immune response, in particular an immune response against *Mycobacterium tuberculosis*.

[0027] The use of a polypeptide comprising:

- [0028] (i) an Rv2386c protein sequence;
- [0029] (ii) a variant of an Rv2386c protein sequence; or
- [0030] (iii) an immunogenic fragment of an Rv2386c protein sequence;

in the manufacture of a medicament for the treatment or prevention of TB, represents another aspect of the invention.

[0031] The present invention provides an isolated polynucleotide comprising a nucleic acid sequence encoding a polypeptide which comprises:

- [0032] (i) an Rv2386c protein sequence;
- [0033] (ii) a variant of an Rv2386c protein sequence; or
- [0034] (iii) an immunogenic fragment of an Rv2386c protein sequence.

[0035] Also provided is a polynucleotide comprising a nucleic acid sequence encoding a polypeptide which comprises:

- [0036] (i) an Rv2386c protein sequence;
- [0037] (ii) a variant of an Rv2386c protein sequence; or
- [0038] (iii) an immunogenic fragment of an Rv2386c protein sequence; for use as a medicament.

[0039] A further aspect of the invention relates to a method for the treatment or prevention of TB comprising the administration of a safe and effective amount of a polynucleotide comprising a nucleic acid sequence encoding a polypeptide comprising:

- [0040] (i) an Rv2386c protein sequence;
- [0041] (ii) a variant of an Rv2386c protein sequence; or
- [0042] (iii) an immunogenic fragment of an Rv2386c protein sequence;

to a subject in need thereof, wherein said polynucleotide induces an immune response, in particular an immune response against *Mycobacterium tuberculosis*.

[0043] Use of a polynucleotide comprising a nucleic acid sequence encoding a polypeptide comprising:

- [0044] (i) an Rv2386c protein sequence;
- [0045] (ii) a variant of an Rv2386c protein sequence; or

[0046] (iii) an immunogenic fragment of an Rv2386c protein sequence;

in the manufacture of a medicament for the treatment or prevention of TB, represents another aspect of the invention.

[0047] Additionally, there is provided a pharmaceutical composition comprising:

[0048] (a) a polypeptide which comprises:

- [0049] (i) an Rv2386c protein sequence;
- [0050] (ii) a variant of an Rv2386c protein sequence; or
- [0051] (iii) an immunogenic fragment of an Rv2386c protein sequence; or

[0052] (b) a polynucleotide comprising a nucleic acid sequence encoding the polypeptide of (a);

[0053] and

[0054] (c) a pharmaceutically acceptable carrier or excipient.

[0055] Further, there is provided an immunogenic composition comprising:

[0056] (a) a polypeptide which comprises:

- [0057] (i) an Rv2386c protein sequence;
- [0058] (ii) a variant of an Rv2386c protein sequence; or
- [0059] (iii) an immunogenic fragment of an Rv2386c protein sequence; or

[0060] (b) a polynucleotide comprising a nucleic acid sequence encoding the polypeptide of (a);

[0061] and

[0062] (c) a non-specific immune response enhancer.

[0063] Suitably the pharmaceutical and immunogenic composition comprise a combination of an Rv2386c and a second heterologous tuberculosis antigen component.

[0064] Also provided is an expression vector comprising a nucleic acid sequence encoding a polypeptide which comprises:

- [0065] (i) an Rv2386c protein sequence;
- [0066] (ii) a variant of an Rv2386c protein sequence; or
- [0067] (iii) an immunogenic fragment of an Rv2386c protein sequence.

[0068] Host cells, transformed with said expression vector, form a further aspect of the invention. Additionally provided is a host cell which recombinantly expresses a polypeptide which comprises:

- [0069] (i) an Rv2386c protein sequence;
- [0070] (ii) a variant of an Rv2386c protein sequence; or
- [0071] (iii) an immunogenic fragment of an Rv2386c protein sequence.

[0072] Further, there is provided a method for the production of a polypeptide comprising:

- [0073] (i) an Rv2386c protein sequence;
- [0074] (ii) a variant of an Rv2386c protein sequence; or
- [0075] (iii) an immunogenic fragment of an Rv2386c protein sequence;

said method comprising the step of recombinantly expressing said polypeptide within a host cell.

[0076] Additionally provided is an antibody or fragment thereof which specifically binds to a polypeptide comprising:

- [0077] (i) an Rv2386c protein sequence;
- [0078] (ii) a variant of an Rv2386c protein sequence; or
- [0079] (iii) an immunogenic fragment of an Rv2386c protein sequence.

[0080] The use of said antibodies in diagnosis is also provided (such as methods for the diagnosis of tuberculosis comprising determining the presence of an antibody or frag-

ment thereof which specifically binds to the polypeptides of the invention in a biological sample from a test subject).

[0081] Also provided are diagnostic kits comprising:

[0082] (a) a polypeptide of the present invention;

[0083] (b) apparatus sufficient to contact said polypeptide with a sample (e.g. whole blood or more suitably PBMC) from an individual; and

[0084] (c) means to quantify the T cell response of the sample.

[0085] Another aspect of the invention relates to a diagnostic kit comprising:

[0086] (a) a polypeptide of the present invention; and

[0087] (b) apparatus sufficient to contact said polypeptide with the dermal cells of a patient.

[0088] In one embodiment the subject receiving a polypeptide, polynucleotide or composition of the invention may have active tuberculosis (e.g. active infection by *M. tuberculosis*). In a second embodiment the subject may have latent tuberculosis (e.g. dormant infection by *M. tuberculosis*). In a third embodiment the subject may be free from tuberculosis (e.g. free from infection by *M. tuberculosis*).

[0089] A subject receiving a polypeptide, polynucleotide or composition of the invention may have previously been vaccinated for tuberculosis (e.g. vaccinated against infection by *M. tuberculosis*), such as having been vaccinated with *Bacillus Calmette-Guerin* (BCG).

[0090] Alternatively, a subject receiving a polypeptide, polynucleotide or composition of the invention may not have previously been vaccinated for tuberculosis (e.g. not vaccinated against infection by *M. tuberculosis*), such as not having been vaccinated with *Bacillus Calmette-Guerin* (BCG).

DESCRIPTION OF THE FIGURES

[0091] FIG. 1: Percentage of CD4 and CD8 cells from immunised CB6F1 mice expressing IFN-gamma and/or IL-2 and/or TNF-alpha cytokines at day 21 (i.e. 7 days post second immunisation).

[0092] FIG. 2: Cytokine profile at day 21 (i.e. 7 days post second immunisation) of the antigen specific CD4 response in immunised CB6F1 mice.

[0093] FIG. 3: Cytokine profile at day 21 (i.e. 7 days post second immunisation) of the antigen specific CD8 response in immunised CB6F1 mice.

[0094] FIG. 4: Percentage of CD4 and CD8 cells from immunised CB6F1 mice expressing IFN-gamma and/or IL-2 and/or TNF-alpha cytokines at day 35 (i.e. 7 days post third immunisation).

[0095] FIG. 5: Cytokine profile at day 35 (i.e. 7 days post third immunisation) of the antigen specific CD4 response in immunised CB6F1 mice.

[0096] FIG. 6: Cytokine profile at day 35 (i.e. 7 days post third immunisation) of the antigen specific CD8 response in immunised CB6F1 mice.

[0097] FIG. 7: Percentage of CD4 and CD8 cells from immunised C57BL/6 mice expressing IFN-gamma and/or IL-2 and/or TNF-alpha cytokines at day 21 (i.e. 7 days post second immunisation).

[0098] FIG. 8: Cytokine profile at day 21 (i.e. 7 days post second immunisation) of the antigen specific CD4 response in immunised C57BL/6 mice.

[0099] FIG. 9: Cytokine profile at day 21 (i.e. 7 days post second immunisation) of the antigen specific CD8 response in immunised C57BL/6 mice.

DESCRIPTION OF THE LISTED SEQUENCES

[0100] SEQ ID No: 1: polypeptide sequence of Rv2386c from *M. tuberculosis* H37Rv strain.

[0101] SEQ ID No: 2: polynucleotide sequence of Rv2386c from *M. tuberculosis* H37Rv strain.

[0102] SEQ ID No: 3: polypeptide sequence of Rv2386c from *M. tuberculosis* CDC1151 strain.

[0103] SEQ ID No: 4: polypeptide sequence of Rv2386c from *M. tuberculosis* F11 strain.

[0104] SEQ ID No: 5: polypeptide sequence of Rv2386c from *M. tuberculosis* Haarlem A strain.

[0105] SEQ ID No: 6: polypeptide sequence of Rv2386c from *M. tuberculosis* C strain.

[0106] SEQ ID No: 7: polypeptide sequence of Rv2386c from BCG.

[0107] SEQ ID No: 8: polypeptide sequence of Mtb8.4.

[0108] SEQ ID No: 9: polypeptide sequence of Mtb9.8.

[0109] SEQ ID No: 10: polypeptide sequence of Mtb9.9.

[0110] SEQ ID No: 11: polypeptide sequence of Ra12.

[0111] SEQ ID No: 12: polypeptide sequence of Ra35.

[0112] SEQ ID No: 13: polypeptide sequence of TbH9.

[0113] SEQ ID No: 14: polypeptide sequence of Mtb40.

[0114] SEQ ID No: 15: polypeptide sequence of Mtb41.

[0115] SEQ ID No: 16: polypeptide sequence of ESAT-6.

[0116] SEQ ID No: 17: polypeptide sequence of Ag85A.

[0117] SEQ ID No: 18: polypeptide sequence of Ag85B.

[0118] SEQ ID No: 19: polypeptide sequence of alpha-crystallin.

[0119] SEQ ID No: 20: polypeptide sequence of MPT64.

[0120] SEQ ID No: 21: polypeptide sequence of Mtb32A.

[0121] SEQ ID No: 22: polypeptide sequence of Ser/Ala mutated mature Mtb32A.

[0122] SEQ ID No: 23: polypeptide sequence of TB10.4.

[0123] SEQ ID No: 24: polypeptide sequence of Mtb72f.

[0124] SEQ ID No: 25: polypeptide sequence of M72.

[0125] SEQ ID No: 26: polypeptide sequence of Mtb71f.

[0126] SEQ ID No: 27: polypeptide sequence of M92 fusion.

[0127] SEQ ID No: 28: polypeptide sequence of M103 fusion.

[0128] SEQ ID No: 29: polypeptide sequence of M114 fusion.

[0129] SEQ ID No: 30: putative human CD4 cell epitope 1.

[0130] SEQ ID No: 31: putative human CD4 cell epitope 2.

[0131] SEQ ID No: 32: putative human CD4 cell epitope 3.

[0132] SEQ ID No: 33: putative human CD4 cell epitope 4.

[0133] SEQ ID No: 34: putative human CD4 cell epitope 5.

[0134] SEQ ID No: 35: putative human CD4 cell epitope 6.

[0135] SEQ ID No: 36: putative human CD4 cell epitope 7.

[0136] SEQ ID No: 37: putative human CD4 cell epitope 8.

[0137] SEQ ID No: 38: putative human CD4 cell epitope 9.

[0138] SEQ ID No: 39: putative human CD4 cell epitope 10.

[0139] SEQ ID No: 40: putative human CD4 cell epitope 11.

[0140] SEQ ID No: 41: putative human CD4 cell epitope 12.

[0141] SEQ ID No: 42: putative human CD4 cell epitope 13.

[0142] 14.	SEQ ID No: 43: putative human CD4 cell epitope	[0179] 29.	SEQ ID No: 81: putative human CD8 cell epitope
[0143] 15.	SEQ ID No: 44: putative human CD4 cell epitope	[0180] 30.	SEQ ID No: 82: putative human CD8 cell epitope
[0144] 16.	SEQ ID No: 45: putative human CD4 cell epitope	[0181] 31.	SEQ ID No: 83: putative human CD8 cell epitope
[0145] 17.	SEQ ID No: 46: putative human CD4 cell epitope	[0182] 32.	SEQ ID No: 84: putative human CD8 cell epitope
[0146] 18.	SEQ ID No: 47: putative human CD4 cell epitope	[0183] 33.	SEQ ID No: 85: putative human CD8 cell epitope
[0147] 19.	SEQ ID No: 48: putative human CD4 cell epitope	[0184] 34.	SEQ ID No: 86: putative human CD8 cell epitope
[0148] 20.	SEQ ID No: 49: putative human CD4 cell epitope	[0185] 35.	SEQ ID No: 87: putative human CD8 cell epitope
[0149] 21.	SEQ ID No: 50: putative human CD4 cell epitope	[0186] 36.	SEQ ID No: 88: putative human CD8 cell epitope
[0150] 22.	SEQ ID No: 51: putative human CD4 cell epitope	[0187] 37.	SEQ ID No: 89: putative human CD8 cell epitope
[0151] 23.	SEQ ID No: 52: putative human CD4 cell epitope	[0188] 38.	SEQ ID No: 90: putative human CD8 cell epitope
[0152]	SEQ ID No: 53: putative human CD8 cell epitope 1.	[0189] 39.	SEQ ID No: 91: putative human CD8 cell epitope
[0153]	SEQ ID No: 54: putative human CD8 cell epitope 2.	[0190]	SEQ ID No: 92: putative human CD8 cell epitope
[0154]	SEQ ID No: 55: putative human CD8 cell epitope 3.	40.	
[0155]	SEQ ID No: 56: putative human CD8 cell epitope 4.	[0191]	SEQ ID No: 93: putative human CD8 cell epitope
[0156]	SEQ ID No: 57: putative human CD8 cell epitope 5.	41.	
[0157]	SEQ ID No: 58: putative human CD8 cell epitope 6.	[0192]	SEQ ID No: 94: putative human CD8 cell epitope
[0158]	SEQ ID No: 59: putative human CD8 cell epitope 7.	42.	
[0159]	SEQ ID No: 60: putative human CD8 cell epitope 8.	[0193]	SEQ ID No: 95: putative human CD8 cell epitope
[0160]	SEQ ID No: 61: putative human CD8 cell epitope 9.	43.	
[0161] 10.	SEQ ID No: 62: putative human CD8 cell epitope	[0194]	SEQ ID No: 96: putative human CD8 cell epitope
[0162] 11.	SEQ ID No: 63: putative human CD8 cell epitope	44.	
[0163]	SEQ ID No: 64: putative human CD8 cell epitope	[0195]	SEQ ID No: 97: putative human CD8 cell epitope
12.		45.	
[0164]	SEQ ID No: 65: putative human CD8 cell epitope	[0196]	SEQ ID No: 98: putative human CD8 cell epitope
13.		46.	
[0165]	SEQ ID No: 66: putative human CD8 cell epitope	[0197]	SEQ ID No: 99: putative human CD8 cell epitope
14.		47.	
[0166]	SEQ ID No: 67: putative human CD8 cell epitope	[0198]	SEQ ID No: 100: putative human CD8 cell epitope
15.		48.	
[0167]	SEQ ID No: 68: putative human CD8 cell epitope	[0199]	SEQ ID No: 101: putative human CD8 cell epitope
16.		49.	
[0168]	SEQ ID No: 69: putative human CD8 cell epitope	[0200]	SEQ ID No: 102: putative human CD8 cell epitope
17.		50.	
[0169]	SEQ ID No: 71: putative human CD8 cell epitope	[0201]	SEQ ID No: 103: putative human CD8 cell epitope
19.		51.	
[0170]	SEQ ID No: 72: putative human CD8 cell epitope	[0202]	SEQ ID No: 104: putative human CD8 cell epitope
20.		52.	
[0171]	SEQ ID No: 73: putative human CD8 cell epitope	[0203]	SEQ ID No: 105: putative human CD8 cell epitope
21.		53.	
[0172]	SEQ ID No: 74: putative human CD8 cell epitope	[0204]	SEQ ID No: 106: putative human CD8 cell epitope
22.		54.	
[0173]	SEQ ID No: 75: putative human CD8 cell epitope	[0205]	SEQ ID No: 108: putative human CD8 cell epitope
23.		56.	
[0174]	SEQ ID No: 76: putative human CD8 cell epitope	[0206]	SEQ ID No: 109: putative human CD8 cell epitope
24.		57.	
[0175]	SEQ ID No: 77: putative human CD8 cell epitope	[0207]	SEQ ID No: 110: putative human CD8 cell epitope
25.		58.	
[0176]	SEQ ID No: 78: putative human CD8 cell epitope	[0208]	SEQ ID No: 111: putative human CD8 cell epitope
26.		59.	
[0177]	SEQ ID No: 79: putative human CD8 cell epitope 27	[0209]	SEQ ID No: 112: putative human CD8 cell epitope
[0178]	SEQ ID No: 80: putative human CD8 cell epitope	60.	
28.		[0210]	SEQ ID No: 113: putative human CD8 cell epitope
		61.	

[0211] SEQ ID No: 114: putative human CD8 cell epitope 62.
 [0212] SEQ ID No: 115: putative human CD8 cell epitope 63.
 [0213] SEQ ID No: 116: putative human CD8 cell epitope 64.
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 [0238] SEQ ID No: 141: putative human CD8 cell epitope 89.
 [0239] SEQ ID No: 142: putative human CD8 cell epitope 90.
 [0240] SEQ ID No: 143: putative human CD8 cell epitope 91.
 [0241] SEQ ID No: 145: putative human CD8 cell epitope 93.
 [0242] SEQ ID No: 146: putative human CD8 cell epitope 94.

[0243] SEQ ID No: 147: putative human CD8 cell epitope 95.
 [0244] SEQ ID No: 148: putative human CD8 cell epitope 96.
 [0245] SEQ ID No: 149: putative human CD8 cell epitope 97.
 [0246] SEQ ID No: 150: putative human CD8 cell epitope 98.
 [0247] SEQ ID No: 151: putative human CD8 cell epitope 99.
 [0248] SEQ ID No: 152: putative human CD8 cell epitope 100.
 [0249] SEQ ID No: 153: putative human CD8 cell epitope 101.
 [0250] SEQ ID No: 154: putative human CD8 cell epitope 102.
 [0251] SEQ ID No: 155: polypeptide sequence of Rv1753c from *M. tuberculosis* H37Rv strain.
 [0252] SEQ ID No: 156: polypeptide sequence of Rv2707c from *M. tuberculosis* H37Rv strain.

DETAILED DESCRIPTION

[0253] Currently, vaccination with live bacteria is the most efficient method for inducing protective immunity. The most common *Mycobacterium* employed for this purpose is *Bacillus Calmette-Guerin* (BCG), an avirulent strain of *M. bovis* which was developed over 60 years ago. However, the safety and efficacy of BCG is a source of controversy—while protecting against severe disease manifestation in children, BCG does not prevent the establishment of latent TB or reactivation of pulmonary disease in adult life. Additionally, some countries, such as the United States, do not vaccinate the general public with this agent.

[0254] Almost all new generation TB vaccines which are currently in clinical development have been designed as pre-exposure vaccines. These include subunit vaccines, which have been particularly effective in boosting immunity induced by prior BCG vaccination, and advanced live mycobacterial vaccines which aim to replace BCG with more efficient and/or safer strains. Although these vaccines aim to improve resistance to infection, they are likely to be less effective as post-exposure or therapeutic vaccines in latent TB cases (Lin M Y et al *Endocrine, Metabolic & Immune Disorders—Drug Targets* 2008 8:15-29).

[0255] Several of the proteins which are strongly expressed during the early stages of *Mycobacterium* infection have been shown to provide strong protective efficacy in animal vaccination models. However, vaccination with antigens which are highly expressed during the early stages of infection may not provide an optimal immune response for dealing with later stages of infection. Adequate control during the later stages of infection may require T cells which are specific for the particular antigens which are expressed at that time.

[0256] Post-exposure vaccines which directly target the dormant persistent bacteria may aid in protecting against TB reactivation, thereby enhancing TB control, or even enabling clearance of the infection. A vaccine targeting latent TB could therefore significantly and economically reduce global TB infection rates.

[0257] Subunit vaccines based on late stage antigens could also be utilised in combination with early stage antigens to provide a multiphase vaccine. Alternatively, late stage antigens could be used to complement and improve BCG vac-

nation (either by boosting the BCG response or through the development of advanced recombinant BCG strains).

[0258] Rv2386c, also known as MbtI, catalyses the initial transformation in mycobactin biosynthesis by converting chorismate to salicylate (Zwahlen et al. *Biochemistry* 2007 46:954-964). Rv2386 has previously been identified as being associated with expression under stress conditions although did not result in protection when administered as a DNA vaccine in a guinea pig model (Vipond et al. *Vaccine* 2006 24:6340-6350).

[0259] Recently, a range of *M. tuberculosis* vaccine candidates have been proposed based on a bioinformatics analysis of the whole genome *M. tuberculosis* genome (Zvi et al. *BMC Medical Genetics* 2008 1:18) and on the testing of differentially expressed proteins in actively and latently infected individuals (Schuck S D et al. *PLoS ONE* 2009 4(5):e5590).

[0260] While macrophages have been shown to act as the principal effectors of *Mycobacterium* immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against tuberculosis is illustrated by the increased rates of TB reactivation in human immunodeficiency virus infected individuals, due to the associated depletion of CD4+ T cells. Furthermore, adoptive transfer of CD4+ T cells taken at the height of the primary immune response to *M. tuberculosis* has been shown to confer protection against *M. tuberculosis* in T cell deficient mice (Orme et al. *J. Exp. Med.* 1983 158:74-83).

[0261] *Mycobacterium*-reactive CD4+ T cells have been shown to be potent producers of γ -interferon (IFN- γ), which, in turn, has been shown to trigger the anti-mycobacterial effects of macrophages in mice (Flynn et al. *J. Exp. Med.* 1993 178:2249-2254). While the role of IFN- γ in humans is less clear, studies have shown that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN- γ or tumor necrosis factor- α , activates human macrophages to inhibit *M. tuberculosis* infection. Furthermore, it is known that IFN- γ stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, interleukin-12 (IL-12) has been shown to play a role in stimulating resistance to *M. tuberculosis* infection. For a review of the immunology of *M. tuberculosis* infection, see Chan & Kaufmann, *Tuberculosis: Pathogenesis, Protection and Control* (Bloom ed., 1994), *Tuberculosis* (2nd ed., Rom and Garay, eds., 2003), and *Harrison's Principles of Internal Medicine*, Chapter 150, pp. 953-966 (16th ed., Braunwald, et al., eds., 2005).

[0262] The present invention relates generally to the identification of Rv2386c as a TB antigen (in particular an antigen associated with latent TB) and to related methods and uses in the prevention and treatment of TB, especially the prevention and treatment of latent TB and the prevention or delay of TB reactivation.

[0263] The invention therefore provides an Rv2386c protein, variant thereof or immunogenic fragment thereof, or a polynucleotide encoding said protein, variant or fragment, for use in the treatment or prevention of TB. Suitably, the use may be specifically in the prevention and treatment of latent TB (especially the treatment of latent TB). Alternatively, the use may be in the prevention or delay of TB reactivation (especially the delay of TB reactivation, for example by a period of months, years or even indefinitely).

[0264] The term "*Mycobacterium* species of the tuberculosis complex" includes those species traditionally considered as causing the disease tuberculosis, as well as *Mycobacterium* environmental and opportunistic species that cause tubercu-

losis and lung disease in immune compromised patients, such as patients with AIDS, e.g., *M. tuberculosis*, *M. bovis*, or *M. africanum*, BCG, *M. avium*, *M. intracellulare*, *M. celatum*, *M. genavense*, *M. haemophilum*, *M. kansasii*, *M. simiae*, *M. vaccae*, *M. fortuitum*, and *M. scrofulaceum* (see, e.g., *Harrison's Principles of Internal Medicine*, Chapter 150, pp. 953-966 (16th ed., Braunwald, et al., eds., 2005)). The present invention is particularly directed to infection with *M. tuberculosis*.

[0265] The term "active infection" refers to an infection (e.g. infection by *M. tuberculosis*) with manifested disease symptoms and/or lesions (suitably with manifested disease symptoms).

[0266] The terms "inactive infection", "dormant infection" or "latent infection" refer to an infection (e.g. infection by *M. tuberculosis*) without manifested disease symptoms and/or lesions (suitably without manifested disease symptoms).

[0267] The term "primary tuberculosis" refers to clinical illness (manifestation of disease symptoms) directly following infection (e.g. infection by *M. tuberculosis*). See, *Harrison's Principles of Internal Medicine*, Chapter 150, pp. 953-966 (16th ed., Braunwald, et al., eds., 2005). The terms "secondary tuberculosis" or "postprimary tuberculosis" refer to the reactivation of a dormant, inactive or latent infection (e.g. infection by *M. tuberculosis*). See, *Harrison's Principles of Internal Medicine*, Chapter 150, pp. 953-966 (16th ed., Braunwald, et al., eds., 2005).

[0268] The term "tuberculosis reactivation" refers to the later manifestation of disease symptoms in an individual that tests positive for infection (e.g. in a tuberculin skin test, suitably in an in vitro T cell based assay) test but does not have apparent disease symptoms. The positive diagnostic test indicates that the individual is infected, however, the individual may or may not have previously manifested active disease symptoms that had been treated sufficiently to bring the tuberculosis into an inactive or latent state. It will be recognised that methods for the prevention, delay or treatment of tuberculosis reactivation can be initiated in an individual manifesting active symptoms of disease.

[0269] The term "drug resistant" tuberculosis refers to an infection (e.g. infection by *M. tuberculosis*) wherein the infecting strain is not held static or killed (i.e. is resistant to) one or more of so-called "front-line" chemotherapeutic agents effective in treating tuberculosis (e.g., isoniazid, rifampin, ethambutol, streptomycin and pyrazinamide).

[0270] The term "multi-drug resistant" tuberculosis refers to an infection (e.g. infection by *M. tuberculosis*) wherein the infecting strain is resistant to two or more of "front-line" chemotherapeutic agents effective in treating tuberculosis.

[0271] A "chemotherapeutic agent" refers to a pharmacological agent known and used in the art to treat tuberculosis (e.g. infection by *M. tuberculosis*). Exemplified pharmacological agents used to treat tuberculosis include, but are not limited to amikacin, aminosalicylic acid, capreomycin, cycloserine, ethambutol, ethionamide, isoniazid, kanamycin, pyrazinamide, rifamycins (i.e., rifampin, rifapentine and rifabutin), streptomycin, ofloxacin, ciprofloxacin, clarithromycin, azithromycin and fluoroquinolones. "First-line" or "Front-line" chemotherapeutic agents used to treat tuberculosis that is not drug resistant include isoniazid, rifampin, ethambutol, streptomycin and pyrazinamide. "Second-line" chemotherapeutic agents used to treat tuberculosis that has demonstrated drug resistance to one or more "first-line" drugs include ofloxacin, ciprofloxacin, ethionamide, aminosalicylic acid, cycloserine, amikacin, kanamycin and capreomy-

cin. Such pharmacological agents are reviewed in Chapter 48 of *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, Hardman and Limbird eds., 2001. The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms also apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Suitably a polypeptide according to the present invention will consist only of naturally occurring amino acid residues, especially those amino acids encoded by the genetic code.

[0272] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogues refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α -carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. Suitably an amino acid is a naturally occurring amino acid or an amino acid analogue, especially a naturally occurring amino acid and in particular those amino acids encoded by the genetic code.

[0273] "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs). Suitably the term "nucleic acid" refers to naturally occurring deoxyribonucleotides or ribonucleotides and polymers thereof.

[0274] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated (suitably it refers to the sequence explicitly indicated). Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka et al., *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini et al., *Mol. Cell. Probes* 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

[0275] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0276] By the term 'Rv2386c protein sequence' as used herein is meant the polypeptide sequence provided in SEQ ID No: 1 or a homologue thereof from a *Mycobacterium* species of the tuberculosis complex, e.g., a species such as *M. tuberculosis*, *M. bovis*, or *M. africanum*, or a *Mycobacterium* species that is environmental or opportunistic and that causes opportunistic infections such as lung infections in immune compromised hosts (e.g., patients with AIDS), e.g., BCG, *M. avium*, *M. intracellulare*, *M. celatum*, *M. genavense*, *M. haemophilum*, *M. kansasii*, *M. simiae*, *M. vaccae*, *M. fortuitum*, and *M. scrofulaceum* (see, e.g., *Harrison's Principles of Internal Medicine*, Chapter 150, pp. 953-966, 16th ed., Braunwald, et al., eds., 2005).

[0277] To ensure a high efficacy rate among vaccinated hosts, the components of a vaccine should be well conserved among the stains of clinical significance. Suitably, the Rv2386c protein is derived from *M. tuberculosis* H37Rv (i.e. the polypeptide sequence provided in SEQ ID No: 1) or a homologue thereof from another *M. tuberculosis* strain (such as CDC1551, F11, Haarlem A and C strains). Strains of *M. tuberculosis* which are associated with drug resistance (e.g. MDR or especially XDR) are a particularly valuable basis for the Rv2386c protein sequence. Strains of interest include:

[0278] CDC1551—transmissible and virulent strain

[0279] Haarlem family (such as Haarlem A)—Drug resistant strains found in crowded human populations. Members of the Haarlem family of *M. tuberculosis* strains have been found in many parts of the world. The first representative of the family was discovered in Haarlem, The Netherlands.

[0280] KZN4207—Drug sensitive isolate from patients in KwaZulu-Natal, South Africa

[0281] KZN1435—Multiple drug resistant (MDR) isolate from patients in KwaZulu-Natal, South Africa

[0282] KZN605—Extensively drug resistant (XDR) isolate from patients in KwaZulu-Natal, South Africa

[0283] C—Highly transmitted in New York City. In one study this strain was found to be more common among injection drug users and resistant to reactive nitrogen intermediates (Friedman et al. *J. Infect. Dis.* 1997 176 (2):478-84)

[0284] 94_M4241A—Isolated in San Francisco in 1994 from a patient born in China. This strain was previously analysed by genomic deletion analysis (Gagneux et al., *PNAS* 2006 103(8):2869-2873).

[0285] 02_1987—Isolated in San Francisco in 2002 from a patient born in South Korea. This strain was previously analyzed by genomic deletion analysis (Gagneux et al., *PNAS* 2006 103(8):2869-2873).

[0286] T92—Isolated in San Francisco in 1999 from a patient born in The Philippines. This strain was published in Hirsh et al. *PNAS* 2004 101:4871-4876).

[0287] T85—Isolated in San Francisco in 1998 from a patient born in China. This strain was published in Hirsh et al. *PNAS* 2004 101:4871-4876).

[0288] EAS054—Isolated in San Francisco in 1993 from a patient born in India. This strain was previously analyzed by genomic deletion analysis (Gagneux et al., *PNAS* 2006 103(8):2869-2873).

[0289] Gagneux et al., *PNAS* 2006 103(8):2869-2873 and Herbert et al. *Infect. Immun.* 2007 75(12):5798-5805 provide valuable background on the range of *M. tuberculosis* strains which are known to exist.

[0290] Most suitably, the Rv2386c protein is selected from the polypeptide sequences provided in SEQ ID Nos: 1 and 3-7, in particular SEQ ID Nos: 1 and 3-6, such as SEQ ID No: 1. Polynucleotides of particular interest are those comprising (such as consisting of) a sequence encoding:

[0291] (i) an Rv2386c protein sequence;

[0292] (ii) a variant of an Rv2386c protein sequence; or

[0293] (iii) an immunogenic fragment of an Rv2386c protein sequence.

[0294] Polynucleotides will suitably comprise (such as consisting of) a variant of SEQ ID NO: 2 or fragment of SEQ ID NO: 2 which encodes an immunogenic fragment of an Rv2386c protein.

Combinations

[0295] The Rv2386c related polypeptides of the present invention can further comprise other components designed to enhance their immunogenicity or to improve these antigens in other respects. For example, improved isolation of the polypeptide antigens may be facilitated through the addition of a stretch of histidine residues (commonly known as a his-tag) towards one end of the antigen.

[0296] The term "his-tag" refers to a string of histidine residues, typically six residues, that are inserted within the reference sequence. To minimise disruption of the activity associated with the reference sequence, a his-tag is typically inserted at the N-terminus, usually immediately after the initiating methionine residue, or else at the C-terminus. They are usually heterologous to the native sequence but are incorporated since they facilitate isolation by improving the protein binding to immobilised metal affinity chromatography resins (IMAC). Generally speaking the presence or absence of a his-tag is not of significance from the point of view of eliciting a desirable immune response against the reference protein. However, to avoid the risk of an adverse reaction against the his-tag itself, it is considered best to minimise the length of the his-tag e.g. to four or fewer residues, in particular two residues (or to exclude the use of a his-tag entirely).

[0297] To improve the magnitude and/or breadth of the elicited immune response the compositions, polypeptides and nucleic acids of the invention can comprise multiple copies of the inventive antigen and/or additional heterologous polypeptides (or polynucleotides encoding them) from *Mycobacterium* species (in particular *M. tuberculosis*).

[0298] One skilled in the art will recognise that when a number of components are utilised in combination, the precise presentation can be varied. For example, a Rv2386c component and an additional copy of the inventive antigen or an additional heterologous antigen component could be presented:

[0299] (1) as two individual polypeptide components;

[0300] (2) as a fusion protein comprising both polypeptide components;

[0301] (3) as one polypeptide and one polynucleotide component;

[0302] (4) as two individual polynucleotide components;

[0303] (5) as a single polynucleotide encoding two individual polypeptide components; or

[0304] (6) as a single polynucleotide encoding a fusion protein comprising both polypeptide components.

[0305] This flexibility applies equally to situations where three or more components are used in combination. However, for convenience, it is often desirable that when a number of components are present they are contained within a single fusion protein or a polynucleotide encoding a single fusion protein. In one embodiment of the invention all antigen components are provided as polypeptides (e.g. within a single fusion protein). In an alternative embodiment of the invention all antigen components are provided as polynucleotides (e.g. a single polynucleotide, such as one encoding a single fusion protein).

[0306] The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

[0307] "Fusion polypeptide" or "fusion protein" refers to a protein having at least two heterologous polypeptides (e.g. at least two *Mycobacterium* sp. polypeptides) covalently linked, either directly or via an amino acid linker. The polypeptides forming the fusion protein are typically linked C-terminus to N-terminus, although they can also be linked C-terminus to C-terminus, N-terminus to N-terminus, or N-terminus to C-terminus. The polypeptides of the fusion protein can be in any order. This term also refers to conservatively modified variants, polymorphic variants, alleles, mutants, immunogenic fragments, and interspecies homologs of the antigens that make up the fusion protein. *Mycobacterium tuberculosis* antigens are described in Cole et al., *Nature* 393:537 (1998), which discloses the entire *Mycobacterium tuberculosis* genome. Antigens from other *Mycobacterium* species that correspond to *M. tuberculosis* antigens can be identified, e.g., using sequence comparison algorithms, as described herein, or other methods known to those of skill in the art, e.g., hybridisation assays and antibody binding assays.

[0308] The term "fused" refers to the covalent linkage between two polypeptides in a fusion protein. The polypeptides are typically joined via a peptide bond, either directly to each other or via an amino acid linker. Optionally, the peptides can be joined via non-peptide covalent linkages known to those of skill in the art.

[0309] Exemplary *M. tuberculosis* antigens which may be combined with Rv2386c include one or more of (e.g. 1 to 5, such as 1 to 3, in particular 1) the following (such as one or more of (i) to (xii)):

[0310] (i) Mtb8.4 (also known as DPV and Rv1174c), the polypeptide sequence of which is described in SEQ ID No: 102 of WO97/09428 (cDNA in SEQ ID No: 101) and in Coler et al *Journal of Immunology* 1998 161: 2356-2364. Of particular interest is the mature Mtb8.4 sequence which is absent the leading signal peptide (i.e. amino acid residues 15-96 from SEQ ID No: 102 of WO97/09428). The full-length polypeptide sequence of Mtb8.4 is shown in SEQ ID No: 8;

[0311] (ii) Mtb9.8 (also known as MSL and Rv0287), the polypeptide sequence of which is described in SEQ ID No: 109 of WO98/53075 (fragments of MSL are disclosed in SEQ ID Nos: 110-124 of WO98/53075, SEQ

- ID Nos: 119 and 120 being of particular interest) and also in Coler et al *Vaccine* 2009 27:223-233 (in particular the reactive fragments shown in FIG. 2 therein). The full-length polypeptide sequence for Mtb9.8 is shown in SEQ ID No: 9;
- [0312] (iii) Mtb9.9 (also known as Mtb9.9A, MTI, MTI-A and Rv1793) the polypeptide sequence of which is described in SEQ ID No: 19 of WO98/53075 and in Alderson et al *Journal of Experimental Medicine* 2000 7:551-559 (fragments of MTI are disclosed in SEQ ID Nos: 17 and 51-66 of WO98/53075, SEQ ID Nos: 17, 51, 52, 53, 56 and 62-65 being of particular interest). A number of polypeptide variants of MTI are described in SEQ ID Nos: 21, 23, 25, 27, 29 and 31 of WO98/53075 and in Alderson et al *Journal of Experimental Medicine* 2000 7:551-559. The full-length polypeptide sequence for Mtb9.9 is shown in SEQ ID No: 10;
- [0313] (iv) Ra12 (also known as Mtb32A C-terminal antigen) the polypeptide sequence of which is described in SEQ ID No: 10 of WO01/98460 and in Skeiky et al *Journal of Immunology* 2004 172:7618-7682. The full-length polypeptide sequence for Ra12 is shown in SEQ ID No: 11;
- [0314] (v) Ra35 (also known as Mtb32A N-terminal antigen) the polypeptide sequence of which is described in SEQ ID No: 8 of WO01/98460 and in Skeiky et al *Journal of Immunology* 2004 172:7618-7682. The full-length polypeptide sequence for Ra35 is shown in SEQ ID No: 12;
- [0315] (vi) TbH9 (also known as Mtb39, Mtb39A, TbH9FL and Rv1196) the polypeptide sequence of which is described in SEQ ID No: 107 of WO97/09428, and also in Dillon et al *Infection and Immunity* 1999 67(6):2941-2950 and Skeiky et al *Journal of Immunology* 2004 172:7618-7682. The full-length polypeptide sequence for TbH9 is shown in SEQ ID No: 13;
- [0316] (vii) Mtb40 (also known as HTCC1 and Rv3616c) the polypeptide sequence of which is described in SEQ ID No: 138 of WO98/53075 (cDNA in SEQ ID No: 137). The full-length polypeptide sequence for Mtb40 is shown in SEQ ID No: 14;
- [0317] (viii) Mtb41 (also known as MTCC2 and Rv0915c) the polypeptide sequence of which is described in SEQ ID No: 142 of WO98/53075 (cDNA in SEQ ID No: 140) and in Skeiky et al *Journal of Immunology* 2000 165:7140-7149. The full-length polypeptide sequence for Mtb41 is shown in SEQ ID No: 15;
- [0318] (ix) ESAT-6 (also known as esxA and Rv3875) the polypeptide sequence of which is described in SEQ ID No: 103 of WO97/09428 (cDNA in SEQ ID No: 104) and in Sorensen et al *Infection and Immunity* 1995 63(5): 1710-1717. The full-length polypeptide sequence for ESAT-6 is shown in SEQ ID No: 16;
- [0319] (x) Ag85 complex antigens (e.g. Ag85A, also known as fbpA and Rv3804c; or Ag85B, also known as fbpB and Rv1886c) which are discussed, for example, in Content et al *Infection and Immunity* 1991 59:3205-3212 and in Huygen et al *Nature Medicine* 1996 2(8): 893-898. The full-length polypeptide sequence for Ag85A is shown in SEQ ID No: 17 (the mature protein of residues 43-338, i.e. lacking the signal peptide, being of particular interest). The full-length polypeptide sequence for Ag85B is shown in SEQ ID No: 18 (the mature protein of residues 41-325, i.e. lacking the signal peptide, being of particular interest);
- [0320] (xi) Alpha-crystallin (also known as hspX and Rv2031c) which is described in Verbon et al *Journal of Bacteriology* 1992 174:1352-1359 and Friscia et al *Clinical and Experimental Immunology* 1995 102:53-57 (of particular interest are the fragments corresponding to residues 71-91, 21-40, 91-110 and 111-130). The full-length polypeptide sequence for alpha-crystallin is shown in SEQ ID No: 19;
- [0321] (xii) Mpt64 (also known as Rv1980c) which is described in Roche et al *Scandinavian Journal of Immunology* 1996 43:662-670. The full-length polypeptide sequence for MPT64 is shown in SEQ ID No: 20 (the mature protein of residues 24-228, i.e. lacking the signal peptide, being of particular interest);
- [0322] (xiii) Mtb32A, the polypeptide sequence of which is described in SEQ ID No: 2 (full-length) and residues 8-330 of SEQ ID No: 4 (mature) of WO01/98460, especially variants having at least one of the catalytic triad mutated (e.g. the catalytic serine residue, which may for example be mutated to alanine). The full-length polypeptide sequence for Mtb32A is shown in SEQ ID No: 21. The mature form of Mtb32A having a Ser/Ala mutation is shown in SEQ ID No: 22;
- [0323] (xiv) TB10.4, the full-length polypeptide sequence for TB10.4 is shown in SEQ ID No: 23;
- [0324] (xv) Rv1753c, the full-length polypeptide sequence for Rv1753c from *Mycobacterium tuberculosis* H37Rv is shown in SEQ ID No: 155; and/or
- [0325] (xvi) Rv2707c, the full-length polypeptide sequence for Rv2707c from *Mycobacterium tuberculosis* H37Rv is shown in SEQ ID No: 156.
- or combinations thereof, such as (for example combinations such as (a) to (g)):
- [0326] (a) a combination of Ra12, TbH9 and Ra35 components, for example in the form of a fusion protein, such as Mtb72f. The polypeptide sequence of Mtb72f is described in SEQ ID No: 6 of WO2006/117240 (cDNA in SEQ ID No: 5) and in Skeiky et al *Journal of Immunology* 2004 172:7618-7682 (where it incorporates an optional His-tag to aid purification, when utilised in the present invention suitably Mtb72f is absent the optional histidine residues). The polypeptide sequence for Mtb72f is shown in SEQ ID No: 24;
- [0327] (b) a combination of Ra12, TbH9 and Ser/Ala mutated Ra35 (i.e. where the catalytic serine residue has been replaced with alanine) components, for example in the form of a fusion protein, such as M72. The polypeptide sequence of M72 is described in SEQ ID No: 4 of WO2006/117240 (cDNA in SEQ ID No: 3) where it incorporates an optional double histidine to aid manufacture, when utilised in the present invention M72 may also incorporate a double histidine though suitably M72 is absent the optional double histidine (i.e. residues 4-725 from SEQ ID No: 4 of WO2006/117240 are of particular interest). The polypeptide sequence for M72 is shown in SEQ ID No: 25;
- [0328] (c) a combination of Mtb8.4, Mtb9.8, Mtb9.9 and Mtb41 components, for example in the form of a fusion protein, such as Mtb71f. The polypeptide sequence of Mtb71f is described in SEQ ID No: 16 of WO99/051748 (cDNA in SEQ ID No: 15), where it incorporates an optional His-tag to aid purification, when utilised in the

present invention suitably Mtb71f corresponds to amino acid residues 9-710 of SEQ ID No: 16 from WO99/051748. The polypeptide sequence for Mtb71f is shown in SEQ ID No: 26;

[0329] (d) a combination of Mtb72f or M72 (suitably without optional histidine residues to aid expression) with Mtb9.8 and Mtb9.9, for example in a fusion protein. The polypeptide sequence for an M72-Mtb9.9-Mtb9.8 fusion is shown in SEQ ID No: 27 (M92 fusion), when used in the present invention, the M72-Mtb9.9-Mtb9.8 fusion may optionally incorporate a double histidine following the initiating methionine residue to aid manufacture;

[0330] (e) a combination of Mtb72f or M72 (suitably without optional histidine residues to aid expression) with Ag85B, for example in a fusion protein, such as Mtb103f. The polypeptide sequence of Mtb103f is described in SEQ ID No: 18 of WO03/070187 (cDNA in SEQ ID No: 10), where it incorporates an optional His-tag to aid purification, when utilised in the present invention suitably Mtb103f corresponds to amino acid residues 8-1016 of SEQ ID No: 18 from WO03/070187. Also of particular interest is M103, i.e. Mtb103f incorporating a Ser/Ala mutation in the Ra35 component, when utilised in the present invention suitably M103 corresponds to amino acid residues 8-1016 of SEQ ID No: 18 from WO03/070187 wherein the Ser residue at position 710 has been replaced with Ala. The polypeptide sequence for M103 is shown in SEQ ID No: 28, when used in the present invention, the M72-Mtb9.9-Mtb9.8 fusion may optionally incorporate a double histidine following the initiating methionine residue to aid manufacture;

[0331] (f) a combination of Mtb72f or M72 (suitably without optional histidine residues to aid expression) with Mtb41, for example in a fusion protein, such as Mtb114f. The polypeptide sequence of Mtb114f is described in SEQ ID No: 16 of WO03/070187 (cDNA in SEQ ID No: 9), where it incorporates an optional His-tag to aid purification, when utilised in the present invention suitably Mtb114f corresponds to amino acid residues 8-1154 of SEQ ID No: 16 from WO03/070187. Also of particular interest is M114, i.e. Mtb114f incorporating a Ser/Ala mutation in the Ra35 component, when utilised in the present invention suitably M114 corresponds to amino acid residues 8-1154 of SEQ ID No: 16 from WO03/070187 wherein the Ser residue at position 710 has been replaced with Ala. The polypeptide sequence for M114 is shown in SEQ ID No: 29, when used in the present invention, the M72-Mtb9.9-Mtb9.8 fusion may optionally incorporate a double histidine following the initiating methionine residue to aid manufacture;

[0332] (g) a combination of Ag85B and ESAT-6 components, such as in a fusion described in Doherty et al *Journal of Infectious Diseases* 2004 190:2146-2153; and/or

[0333] (h) a combination of Ag85B and TB10.4 components, such as in a fusion described in Dietrich et al *Journal of Immunology* 2005 174(10):6332-6339 190: 2146-2153.

[0334] Combinations of an Rv2386c component and an Mtb40 component are of particular interest. Obviously such combinations could optionally contain other additional antigen components (e.g. an M72 component).

[0335] Another combination of interest comprises an Rv2386c component and an M72 component.

[0336] A further combination of interest comprises an Rv2386c component and an Rv1753c component.

[0337] Other combinations of interest include those comprising an Rv2386c component and an Rv2707c component.

[0338] An additional combination of interest comprises an Rv2386c component and an alpha-crystallin component.

[0339] The skilled person will recognise that combinations need not rely upon the specific sequences described in above in (i)-(xvi) and (a)-(h), and that conservatively modified variants (e.g. having at least 70% identity, such as at least 80% identity, in particular at least 90% identity and especially at least 95% identity) or immunogenic fragments (e.g. at least 20% of the full length antigen, such as at least 50% of the antigen, in particular at least 70% and especially at least 80%) of the described sequences can be used to achieve the same practical effect.

[0340] Each of the above individual antigen sequences is also disclosed in Cole et al *Nature* 1998 393:537-544 and Camus *Microbiology* 2002 148:2967-2973. The genome of *M. tuberculosis* H37Rv is publicly available, for example at the Wellcome Trust Sanger Institute website (www.sanger.ac.uk/Projects/M_tuberculosis/) and elsewhere.

[0341] Many of the above antigens are also disclosed in U.S. patent application Ser. Nos. 08/523,435, 08/523,436, 08/658,800, 08/659,683, 08/818,111, 08/818,112, 08/942,341, 08/942,578, 08/858,998, 08/859,381, 09/056,556, 09/072,596, 09/072,967, 09/073,009, 09/073,010, 09/223,040, 09/287,849 and in PCT patent applications PCT/US98/10407, PCT/US98/10514, PCT/US99/03265, PCT/US99/03268, PCT/US99/07717, WO97/09428 and WO97/09429, WO98/16645, WO98/16646, each of which is herein incorporated by reference.

[0342] The compositions, polypeptides, and nucleic acids of the invention can also comprise additional polypeptides from other sources. For example, the compositions and fusion proteins of the invention can include polypeptides or nucleic acids encoding polypeptides, wherein the polypeptide enhances expression of the antigen, e.g., NS1, an influenza virus protein (see, e.g. WO99/40188 and WO93/04175). The nucleic acids of the invention can be engineered based on codon preference in a species of choice, e.g., humans (in the case of in vivo expression) or a particular bacterium (in the case of polypeptide production).

[0343] The Rv2386c component may also be administered with one or more chemotherapeutic agents effective against tuberculosis (e.g. *M. tuberculosis* infection). Examples of such chemotherapeutic agents include, but are not limited to, amikacin, aminosalicic acid, capreomycin, cycloserine, ethambutol, ethionamide, isoniazid, kanamycin, pyrazinamide, rifamycins (i.e., rifampin, rifapentine and rifabutin), streptomycin, ofloxacin, ciprofloxacin, clarithromycin, azithromycin and fluoroquinolones. Such chemotherapy is determined by the judgment of the treating physician using preferred drug combinations. "First-line" chemotherapeutic agents used to treat tuberculosis (e.g. *M. tuberculosis* infection) that is not drug resistant include isoniazid, rifampin, ethambutol, streptomycin and pyrazinamide. "Second-line" chemotherapeutic agents used to treat tuberculosis (e.g. *M. tuberculosis* infection) that has demonstrated drug resistance to one or more "first-line" drugs include ofloxacin, ciprofloxacin, ethionamide, aminosalicic acid, cycloserine, amikacin, kanamycin and capreomycin.

[0344] Conventional chemotherapeutic agents are generally administered over a relatively long period (ca. 9 months). Combination of conventional chemotherapeutic agents with the administration of a Rv2386c component according to the present invention may enable the chemotherapeutic treatment period to be reduced (e.g. to 8 months, 7 months, 6 months, 5 months, 4 months, 3 months or less) without a decrease in efficacy.

[0345] Of particular interest is the use of an Rv2386c component in conjunction with *Bacillus Calmette-Guerin* (BCG). For example, in the form of a modified BCG which recombinantly expresses Rv2386c (or a variant or fragment thereof as described herein). Alternatively, the Rv2386c component may be used to enhance the response of a subject to BCG vaccination, either by co-administration or by boosting a previous BCG vaccination. When used to enhance the response of a subject to BCG vaccination, the Rv2386c component may obviously be provided in the form of a polypeptide or a polynucleotide (optionally in conjunction with additional antigenic components as described above).

[0346] The skilled person will recognise that combinations of components need not be administered together and may be applied: separately or in combination; at the same time, sequentially or within a short period; though the same or through different routes. Nevertheless, for convenience it is generally desirable (where administration regimes are compatible) to administer a combination of components as a single composition.

[0347] The polypeptides, polynucleotides and compositions of the present invention will usually be administered to humans, but are effective in other mammals including domestic mammals (e.g., dogs, cats, rabbits, rats, mice, guinea pigs, hamsters, chinchillas) and agricultural mammals (e.g., cows, pigs, sheep, goats, horses).

Immunogenic Fragments

[0348] T cell epitopes are short contiguous stretches of amino acids which are recognised by T cells (e.g. CD4+ or CD8+ T cells). Identification of T cell epitopes may be achieved through epitope mapping experiments which are well known to the person skilled in the art (see, for example, Paul, *Fundamental Immunology*, 3rd ed., 243-247 (1993); Beißbarth et al *Bioinformatics* 2005 21(Suppl. 1):i29-i37).

[0349] Alternatively, epitopes may be predicted using the approaches discussed in the Examples. As a result of the crucial involvement of the T cell response in tuberculosis, it is readily apparent that fragments of the full length Rv2386c polypeptide which contain at least one T cell epitope will be immunogenic and may contribute to immunoprotection. Such fragments are referred to herein as immunogenic fragments.

[0350] Immunogenic fragments according to the present invention will typically comprise at least 9 contiguous amino acids from the full length polypeptide sequence (e.g. at least 10), such as at least 12 contiguous amino acids (e.g. at least 15 or at least 20 contiguous amino acids), in particular at least 50 contiguous amino acids, such as at least 100 contiguous amino acids (for example at least 200 contiguous amino acids). Suitably the immunogenic fragments will be at least 20%, such as at least 50%, at least 70% or at least 80% of the length of the full length polypeptide sequence.

[0351] It will be understood that in a diverse out-bred population, such as humans, different HLA types mean that specific epitopes may not be recognised by all members of the

population. Consequently, to maximise the level of recognition and scale of immune response to a polypeptide, it is generally desirable that an immunogenic fragment contains a plurality of the epitopes from the full length sequence (suitably all epitopes).

[0352] Particular fragments of the Rv2386c protein which may be of use include those containing at least one CD4+ epitope, suitably at least two CD4+ epitopes and especially all CD4+ epitopes (such as those epitopes described in the Examples and in SEQ ID Nos: 30-52, particularly those associated with a plurality of HLA alleles, e.g. those associated with 2, 3, 4, 5 or more alleles).

[0353] Other fragments of the Rv2386c protein which may be of use include those containing at least one CD8 epitope, suitably at least two CD8 epitopes and especially all CD8 epitopes (such as those epitopes described in the Examples and in SEQ ID Nos: 53-154, particularly those associated with a plurality of HLA alleles, e.g. those associated with 2, 3, 4, 5 or more alleles).

[0354] Where an individual fragment of the full length polypeptide is used, such a fragment is considered to be immunogenic where it elicits a response which is at least 20%, suitably at least 50% and especially at least 75% (such as at least 90%) of the activity of the reference sequence in an in vitro restimulation assay of PBMC or whole blood with specific antigens (e.g. restimulation for a period of between several hours to up to two weeks, such as up to one day, 1 day to 1 week or 1 to 2 weeks) that measures the activation of the cells via lymphoproliferation, production of cytokines in the supernatant of culture (measured by ELISA, CBA etc) or characterisation of T and B cell responses by intra and extracellular staining (e.g. using antibodies specific to immune markers, such as CD3, CD4, CD8, IL2, TNF α , IFN γ , CD40L, CD69 etc) followed by analysis with a flowcytometer. Suitably, a fragment is considered to be immunogenic where it elicits a response which is at least 20%, suitably at least 50% and especially at least 75% (such as at least 90%) of the activity of the reference sequence in a T cell proliferation and/or IFN-gamma production assay.

[0355] In some circumstances a plurality of fragments of the full length polypeptide (which may or may not be overlapping and may or may not cover the entirety of the full length sequence) may be used to obtain an equivalent biological response to the full length sequence itself. For example, at least two immunogenic fragments (such as three, four or five) as described above, which in combination provide at least 50%, suitably at least 75% and especially at least 90% activity of the reference sequence in an in vitro restimulation assay of PBMC or whole blood (e.g. a T cell proliferation and/or IFN-gamma production assay).

Variants

[0356] "Variants" or "conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences.

[0357] Due to the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be

altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations lead to “silent” or “degenerate” variants, which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognise that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[0358] A polynucleotide of the invention may contain a number of silent variations (for example, 1-50, such as 1-25, in particular 1-5, and especially 1 codon(s) may be altered) when compared to the reference sequence. A polynucleotide of the invention may contain a number of non-silent conservative variations (for example, 1-50, such as 1-25, in particular 1-5, and especially 1 codon(s) may be altered) when compared to the reference sequence. Non-silent variations are those which result in a change in the encoded amino acid sequence (either through the substitution, deletion or addition of amino acid residues). Those skilled in the art will recognise that a particular polynucleotide sequence may contain both silent and non-silent conservative variations.

[0359] In respect of variants of a protein sequence, the skilled person will recognise that individual substitutions, deletions or additions to polypeptide, which alters, adds or deletes a single amino acid or a small percentage of amino acids is a “conservatively modified variant” where the alteration(s) results in the substitution of an amino acid with a functionally similar amino acid or the substitution/deletion/addition of residues which do not substantially impact the biological function of the variant.

[0360] Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0361] A polypeptide of the invention may contain a number of conservative substitutions (for example, 1-50, such as 1-25, in particular 1-10, and especially 1 amino acid residue (s) may be altered) when compared to the reference sequence. In general, such conservative substitutions will fall within one of the amino-acid groupings specified below, though in some circumstances other substitutions may be possible without substantially affecting the immunogenic properties of the antigen. The following eight groups each contain amino acids that are typically conservative substitutions for one another:

- [0362]** 1) Alanine (A), Glycine (G);
 - [0363]** 2) Aspartic acid (D), Glutamic acid (E);
 - [0364]** 3) Asparagine (N), Glutamine (Q);
 - [0365]** 4) Arginine (R), Lysine (K);
 - [0366]** 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
 - [0367]** 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
 - [0368]** 7) Serine (S), Threonine (T); and
 - [0369]** 8) Cysteine (C), Methionine (M)
- [0370]** (see, e.g., Creighton, *Proteins* 1984).

[0371] Suitably such substitutions do not occur in the region of an epitope, and do not therefore have a significant impact on the immunogenic properties of the antigen.

[0372] Protein variants may also include those wherein additional amino acids are inserted compared to the reference sequence, for example, such insertions may occur at 1-10 locations (such as 1-5 locations, suitably 1 or 2 locations, in particular 1 location) and may, for example, involve the addition of 50 or fewer amino acids at each location (such as 20 or fewer, in particular 10 or fewer, especially 5 or fewer). Suitably such insertions do not occur in the region of an epitope, and do not therefore have a significant impact on the immunogenic properties of the antigen. One example of insertions includes a short stretch of histidine residues (e.g. 2-6 residues) to aid expression and/or purification of the antigen in question.

[0373] Protein variants include those wherein amino acids have been deleted compared to the reference sequence, for example, such deletions may occur at 1-10 locations (such as 1-5 locations, suitably 1 or 2 locations, in particular 1 location) and may, for example, involve the deletion of 50 or fewer amino acids at each location (such as 20 or fewer, in particular 10 or fewer, especially 5 or fewer). Suitably such deletions do not occur in the region of an epitope, and do not therefore have a significant impact on the immunogenic properties of the antigen.

[0374] The skilled person will recognise that a particular protein variant may comprise substitutions, deletions and additions (or any combination thereof).

[0375] Methods of determining the epitope regions of an antigen are described and exemplified in the Examples.

[0376] Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity (such as at least about 95%, at least about 98% or at least about 99%) to the associated reference sequence.

[0377] The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or sub-sequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., 70% identity, optionally 75%, 80%, 85%, 90%, 95%, 98% or 99% identity over a specified region), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be “substantially identical.” This definition also refers to the complement of a test sequence. Optionally, the identity exists over a region that is at least about 25 to about 50 amino acids or nucleotides in length, or optionally over a region that is 75-100 amino acids or nucleotides in length. Suitably, the comparison is performed over a window corresponding to the entire length of the reference sequence.

[0378] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0379] A “comparison window”, as used herein, refers to a segment in which a sequence may be compared to a

reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by manual alignment and visual inspection (see, e.g., *Current Protocols in Molecular Biology* (Ausubel et al., eds. 1995 supplement)).

[0380] One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendrogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360 (1987). The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153 (1989). The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. Using PILEUP, a reference sequence is compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. PILEUP can be obtained from the GCG sequence analysis software package, e.g., version 7.0 (Devereaux et al., *Nuc. Acids Res.* 12:387-395 (1984)).

[0381] Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., *Nuc. Acids Res.* 25:3389-3402 (1997) and Altschul et al., *J. Mol. Biol.* 215:403-410 (1990), respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (website at www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul et al., supra). These initial neighbourhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of

matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLO-SUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0382] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0383] The present invention also extends to polynucleotides comprising a first nucleotide sequence which selectively hybridises under moderately stringent conditions (such as under highly stringent conditions) to the complement of a second nucleotide sequence which encodes a polypeptide comprising:

[0384] (i) an Rv2386c protein sequence;

[0385] (ii) a variant of an Rv2386c protein sequence; or

[0386] (iii) an immunogenic fragment of an Rv2386c protein sequence.

[0387] The phrase "highly stringent hybridisation conditions" refers to conditions under which a probe will hybridise to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Highly stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridise specifically at higher temperatures. An extensive guide to the hybridisation of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Probes*, "Overview of principles of hybridisation and the strategy of nucleic acid assays" (1993). Generally, highly stringent conditions are selected to be about 5-10° C. lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridise to the target sequence at equilibrium (as the target sequences are present in excess, at T_m , 50% of the probes are occupied at equilibrium). Highly stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C. for short probes (e.g., 10 to 50 nucle-

otides) and at least about 60° C. for long probes (e.g., greater than 50 nucleotides). Highly stringent conditions may also be achieved with the addition of destabilising agents such as formamide. For selective or specific hybridisation, a positive signal is at least two times background, optionally 10 times background hybridisation.

[0388] Exemplary highly stringent hybridisation conditions can be as following: 50% formamide, 5×SSC, and 1% SDS, incubating at 42° C., or, 5×SSC, 1% SDS, incubating at 65° C., with wash in 0.2×SSC, and 0.1% SDS at 65° C.

[0389] Nucleic acids that do not hybridise to each other under highly stringent conditions are still functionally equivalent if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridise under moderately stringent hybridisation conditions.

[0390] Exemplary “moderately stringent hybridisation conditions” include a hybridisation in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37° C., and a wash in 1×SSC at 45° C. A positive hybridisation is at least twice background. Those of ordinary skill will readily recognise that alternative hybridisation and wash conditions can be utilised to provide conditions of similar stringency.

[0391] The phrase “selectively (or specifically) hybridises to” refers to the binding, duplexing, or hybridising of a molecule only to a particular nucleotide sequence under stringent hybridisation conditions when that sequence is present in a complex mixture (e.g., total cellular or library DNA or RNA).

[0392] In any event, variants of a polypeptide sequence will have essentially the same activity as the reference sequence (in the case of polynucleotides, variant polynucleotide sequences will encode a polypeptide which has essentially the same activity as the reference sequence). By essentially the same activity is meant at least 50%, suitably at least 75% and especially at least 90% activity of the reference sequence in an in vitro restimulation assay of PBMC or whole blood with specific antigens (e.g. restimulation for a period of between several hours to up to two weeks, such as up to one day, 1 day to 1 week or 1 to 2 weeks) that measures the activation of the cells via lymphoproliferation, production of cytokines in the supernatant of culture (measured by ELISA, CBA etc) or characterisation of T and B cell responses by intra and extracellular staining (e.g. using antibodies specific to immune markers, such as CD3, CD4, CD8, IL2, TNF α , IFN γ , CD40L, CD69 etc) followed by analysis with a flow-cytometer. Suitably, by essentially the same activity is meant at least 50%, suitably at least 75% and especially at least 90% activity of the reference sequence in a T cell proliferation and/or IFN-gamma production assay

Polynucleotide Compositions

[0393] As used herein, the term “polynucleotide” refers to a molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a polynucleotide encoding a polypeptide refers to a polynucleotide segment that contains one or more coding sequences yet is substantially isolated away from, or purified free from, total genomic DNA of the species from which the polynucleotide is obtained.

[0394] As will be understood by those skilled in the art, the polynucleotides of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be

adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

[0395] “Isolated,” as used herein, means that a polynucleotide is substantially away from other coding sequences, and that the polynucleotide does not contain large portions of unrelated coding DNA, such as large chromosomal fragments or other functional genes or polypeptide coding regions. An isolated nucleic acid is separated from other open reading frames that flank the gene and encode proteins other than the gene. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

[0396] As will be recognised by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

[0397] Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes a *Mycobacterium* antigen or a portion thereof) or may comprise a variant, or a biological or functional equivalent of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions, as further described below, preferably such that the immunogenicity of the encoded polypeptide is not diminished, relative to the reference protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein.

[0398] In additional embodiments, the present invention provides isolated polynucleotides and polypeptides comprising various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed herein. For example, polynucleotides are provided by this invention that comprise at least about 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of the reference sequence disclosed herein as well as all intermediate lengths there between. It will be readily understood that “intermediate lengths”, in this context, means any length between the quoted values, such as 30, 31, 32, etc.; 50, 51, 52, 53, etc.; 100, 101, 102, 103, etc.; 150, 151, 152, 153, etc.; including all integers through 200-500; 500-1,000, and the like.

[0399] Moreover, it will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear relatively low identity to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention, for example polynucleotides that are optimized for human and/or primate codon selection. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or func-

tion. Alleles may be identified using standard techniques (such as hybridisation, amplification and/or database sequence comparison).

Polynucleotide Identification and Characterisation

[0400] Polynucleotides may be identified, prepared and/or manipulated using any of a variety of well established techniques. For example, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs. Such screens may be performed, for example, using a Synteni microarray (Palo Alto, Calif.) according to the manufacturer's instructions (and essentially as described by Schena et al., *Proc. Natl. Acad. Sci. USA* 93:10614-10619 (1996) and Heller et al., *Proc. Natl. Acad. Sci. USA* 94:2150-2155 (1997)). Alternatively, polynucleotides may be amplified from cDNA prepared from cells expressing the proteins described herein, such as *M. tuberculosis* cells. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesised.

[0401] An amplified portion of a polynucleotide may be used to isolate a full length gene from a suitable library (e.g., a *M. tuberculosis* cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

[0402] For hybridisation techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with ³²P) using well known techniques. A bacterial or bacteriophage library is then generally screened by hybridising filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (see Sambrook et al., *Molecular Cloning: A Laboratory Manual* (2000)). Hybridising colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences can then be assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

[0403] Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68° C. to 72° C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

[0404] One such amplification technique is inverse PCR (see Triglia et al., *Nucl. Acids Res.* 16:8186 (1988)), which

uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularised by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridises to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom et al., *PCR Methods Applic.* 1:111-19 (1991)) and walking PCR (Parker et al., *Nucl. Acids. Res.* 19:3055-60 (1991)). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

[0405] In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (e.g., NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences may also be obtained by analysis of genomic fragments.

Polynucleotide Expression in Host Cells

[0406] Polynucleotide sequences or fragments thereof which encode polypeptides, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of a polypeptide in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

[0407] As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

[0408] Moreover, the polynucleotide sequences can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

[0409] Natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of polypeptide activity, it may be useful to encode a chimeric protein that can be recognised by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the polypeptide-encoding sequence and the heterologous protein sequence, so that the polypeptide may be cleaved and purified away from the heterologous moiety.

[0410] Sequences encoding a desired polypeptide may be synthesised, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. et al., *Nucl. Acids Res. Symp. Ser.* pp. 215-223 (1980), Horn et al., *Nucl. Acids Res. Symp. Ser.* pp. 225-232 (1980)). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge et al., *Science* 269:202-204 (1995)) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo Alto, Calif.).

[0411] A newly synthesised peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, *Proteins, Structures and Molecular Principles* (1983)) or other comparable techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

[0412] In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook et al., *Molecular Cloning, A Laboratory Manual* (2000), and Ausubel et al., *Current Protocols in Molecular Biology* (updated annually).

[0413] A variety of expression vector/host systems may be utilised to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

[0414] The “control elements” or “regulatory sequences” present in an expression vector are those non-translated regions of the vector—enhancers, promoters, 5' and 3' untranslated regions—which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the

vector system and host utilised, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the PBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or PSPORT1 plasmid (Gibco BRL, Gaithersburg, Md.) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

[0415] In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the expressed polypeptide. For example, when large quantities are needed, for example for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding the polypeptide of interest may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke & Schuster, *J. Biol. Chem.* 264:5503-5509 (1989)); and the like. pGEX Vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

[0416] In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. Other vectors containing constitutive or inducible promoters include GAP, PGK, GAL and ADH. For reviews, see Ausubel et al. (supra) and Grant et al., *Methods Enzymol.* 153:516-544 (1987) and Romas et al. *Yeast* 8 423-88 (1992).

[0417] In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J.* 6:307-311 (1987)). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi et al., *EMBO J.* 3:1671-1680 (1984); Broglie et al., *Science* 224:838-843 (1984); and Winter et al., *Results Probl. Cell Differ.* 17:85-105 (1991)). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, e.g., Hobbs in *McGraw Hill Yearbook of Science and Technology* pp. 191-196 (1992)).

[0418] An insect system may also be used to express a polypeptide of interest. For example, in one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia larvae*. The sequences encoding the polypeptide may be cloned into a non-essential

region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, *S. frugiperda* cells or *Trichoplusia larvae* in which the polypeptide of interest may be expressed (Engelhard et al., *Proc. Natl. Acad. Sci. U.S.A.* 91:3224-3227 (1994)).

[0419] In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. U.S.A.* 81:3655-3659 (1984)). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. Methods and protocols for working with adenovirus vectors are reviewed in Wold, *Adenovirus Methods and Protocols*, 1998. Additional references regarding use of adenovirus vectors can be found in *Adenovirus: A Medical Dictionary, Bibliography, and Annotated Research Guide to Internet References*, 2004.

[0420] Specific initiation signals may also be used to achieve more efficient translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, et al., *Results Probl. Cell Differ.* 20:125-162 (1994)).

[0421] In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and W138, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

[0422] For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same

or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

[0423] Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler et al., *Cell* 11:223-32 (1977)) and adenine phosphoribosyltransferase (Lowy et al., *Cell* 22:817-23 (1990)) genes which can be employed in tk.sup.- or aprt.sup.- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler et al., *Proc. Natl. Acad. Sci. U.S.A.* 77:3567-70 (1980)); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin et al., *J. Mol. Biol.* 150:1-14 (1981)); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilise indole in place of tryptophan, or hisD, which allows cells to utilise histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. U.S.A.* 85:8047-51 (1988)). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, β -glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes et al., *Methods Mol. Biol.* 55:121-131 (1995)).

[0424] Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding a polypeptide is inserted within a marker gene sequence, recombinant cells containing sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a polypeptide-encoding sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

[0425] Alternatively, host cells which contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridisations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

[0426] A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilising monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and

other assays are described, among other places, in Hampton et al., *Serological Methods, a Laboratory Manual* (1990) and Maddox et al., *J. Exp. Med.* 158:1211-1216 (1983).

[0427] A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labelled hybridisation or PCR probes for detecting sequences related to polynucleotides include oligolabeling, nick translation, end-labelling or PCR amplification using a labelled nucleotide. Alternatively, the sequences, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

[0428] Host cells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides may be designed to contain signal sequences which direct secretion of the encoded polypeptide through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAG extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, Calif.) between the purification domain and the encoded polypeptide may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilised metal ion affinity chromatography) as described in Porath et al., *Prot. Exp. Purif.* 3:263-281 (1992) while the enterokinase cleavage site provides a means for purifying the desired polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll et al., *DNA Cell Biol.* 12:441-453 (1993).

In Vivo Polynucleotide Delivery Techniques

[0429] In additional embodiments, genetic constructs comprising one or more of the polynucleotides of the invention are introduced into cells in vivo. This may be achieved using any of a variety of well known approaches, several of which are outlined below for the purpose of illustration.

[0430] 1. Adenovirus

[0431] One of the preferred methods for in vivo delivery of one or more nucleic acid sequences involves the use of an

adenovirus expression vector. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to express a polynucleotide that has been cloned therein in a sense or antisense orientation. Of course, in the context of an antisense construct, expression does not require that the gene product be synthesised.

[0432] The expression vector comprises a genetically engineered form of an adenovirus. Knowledge of the genetic organisation of adenovirus, a 36 kb, linear, double-stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus & Horwitz, 1992). In contrast to retrovirus, the adenoviral infection of host cells does not result in chromosomal integration because adenoviral DNA can replicate in an episomal manner without potential genotoxicity. Also, adenoviruses are structurally stable, and no genome rearrangement has been detected after extensive amplification. Adenovirus can infect virtually all epithelial cells regardless of their cell cycle stage. So far, adenoviral infection appears to be linked only to mild disease such as acute respiratory disease in humans.

[0433] Adenovirus is particularly suitable for use as a gene transfer vector because of its mid-sized genome, ease of manipulation, high titre, wide target-cell range and high infectivity. Both ends of the viral genome contain 100-200 base pair inverted repeats (ITRs), which are cis elements necessary for viral DNA replication and packaging. The early (E) and late (L) regions of the genome contain different transcription units that are divided by the onset of viral DNA replication. The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome and a few cellular genes. The expression of the E2 region (E2A and E2B) results in the synthesis of the proteins for viral DNA replication. These proteins are involved in DNA replication, late gene expression and host cell shut-off (Renan, 1990). The products of the late genes, including the majority of the viral capsid proteins, are expressed only after significant processing of a single primary transcript issued by the major late promoter (MLP). The MLP, (located at 16.8 m.u.) is particularly efficient during the late phase of infection, and all the mRNA's issued from this promoter possess a 5'-tripartite leader (TPL) sequence which makes them preferred mRNA's for translation.

[0434] In a current system, recombinant adenovirus is generated from homologous recombination between shuttle vector and provirus vector. Due to the possible recombination between two proviral vectors, wild-type adenovirus may be generated from this process. Therefore, it is critical to isolate a single clone of virus from an individual plaque and examine its genomic structure.

[0435] Generation and propagation of the current adenovirus vectors, which are replication deficient, depend on a unique helper cell line, designated 293, which was transformed from human embryonic kidney cells by Ad5 DNA fragments and constitutively expresses E1 proteins (Graham et al., 1977). Since the E3 region is dispensable from the adenovirus genome (Jones & Shenk, 1978), the current adenovirus vectors, with the help of 293 cells, carry foreign DNA in either the E1, the D3 or both regions (Graham & Prevec, 1991). In nature, adenovirus can package approximately 105% of the wild-type genome (Ghosh-Choudhury et al., 1987), providing capacity for about 2 extra kb of DNA. Combined with the approximately 5.5 kb of DNA that is replaceable in the E1 and E3 regions, the maximum capacity

of the current adenovirus vector is under 7.5 kB, or about 15% of the total length of the vector. More than 80% of the adenovirus viral genome remains in the vector backbone and is the source of vector-borne cytotoxicity. Also, the replication deficiency of the E1-deleted virus is incomplete. For example, leakage of viral gene expression has been observed with the currently available vectors at high multiplicities of infection (MOI) (Mulligan, 1993).

[0436] Helper cell lines may be derived from human cells such as human embryonic kidney cells, muscle cells, hematopoietic cells or other human embryonic mesenchymal or epithelial cells. Alternatively, the helper cells may be derived from the cells of other mammalian species that are permissive for human adenovirus. Such cells include, e.g., Vero cells or other monkey embryonic mesenchymal or epithelial cells. As stated above, the currently preferred helper cell line is 293.

[0437] Rancher et al. (1995) have disclosed improved methods for culturing 293 cells and propagating adenovirus. In one format, natural cell aggregates are grown by inoculating individual cells into 1 litre siliconised spinner flasks (Technique, Cambridge, UK) containing 100-200 ml of medium. Following stirring at 40 rpm, the cell viability is estimated with trypan blue. In another format, Fibra-Cel microcarriers (Bibby Sterlin, Stone, UK) (5 g/l) is employed as follows. A cell inoculum, resuspended in 5 ml of medium, is added to the carrier (50 ml) in a 250 ml Erlenmeyer flask and left stationary, with occasional agitation, for 1 to 4 h. The medium is then replaced with 50 ml of fresh medium and shaking initiated. For virus production, cells are allowed to grow to about 80% confluence, after which time the medium is replaced (to 25% of the final volume) and adenovirus added at an MOI of 0.05. Cultures are left stationary overnight, following which the volume is increased to 100% and shaking commenced for another 72 h.

[0438] Other than the requirement that the adenovirus vector be replication defective, or at least conditionally defective, the nature of the adenovirus vector is not believed to be crucial to the successful practice of the invention. The adenovirus may be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material in order to obtain a conditional replication-defective adenovirus vector for use in the present invention, since Adenovirus type 5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector.

[0439] As stated above, the typical vector according to the present invention is replication defective and will not have an adenovirus E1 region. Thus, it will be most convenient to introduce the polynucleotide encoding the gene of interest at the position from which the E1-coding sequences have been removed. However, the position of insertion of the construct within the adenovirus sequences is not critical to the invention. The polynucleotide encoding the gene of interest may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described by Karlsson et al. (1986) or in the E4 region where a helper cell line or helper virus complements the E4 defect.

[0440] Adenovirus is easy to grow and manipulate and exhibits broad host range in vitro and in vivo. This group of viruses can be obtained in high titres, e.g., 10^9 - 10^{11} plaque-forming units per ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vec-

tors are episomal and, therefore, have low genotoxicity to host cells. No side effects have been reported in studies of vaccination with wild-type adenovirus (Couch et al., 1963; Top et al., 1971), demonstrating their safety and therapeutic potential as in vivo gene transfer vectors.

[0441] Adenovirus vectors have been used in eukaryotic gene expression (Levrero et al., 1991; Gomez-Foix et al., 1992) and vaccine development (Grunhaus & Horwitz, 1992; Graham & Prevec, 1992). Recently, animal studies suggested that recombinant adenovirus could be used for gene therapy (Stratford-Perricaudet & Perricaudet, 1991; Stratford-Perricaudet et al., 1990; Rich et al., 1993). Studies in administering recombinant adenovirus to different tissues include trachea instillation (Rosenfeld et al., 1991; Rosenfeld et al., 1992), muscle injection (Ragot et al., 1993), peripheral intravenous injections (Herz & Gerard, 1993) and stereotactic inoculation into the brain (Le Gal La Salle et al., 1993).

[0442] Adenovirus vectors may originate from human adenovirus. Alternatively they may originate from adenovirus of other species e.g. chimpanzee which may have the advantage that the viral vectors are not neutralised by antibodies against human adenovirus circulating in many human subjects (see e.g.: Tatsis N et al *Gene Therapy* 2006 13:421-429).

[0443] Adenovirus type 35, which is relatively uncommon and therefore there are low levels of pre-existing immunity to the vector itself, has been used as a delivery system in certain tuberculosis vaccines which are being developed (see for example, Radosevic et al *Infection and Immunity* 2007 75(8): 4105-4115). Adenovirus type 35 may also be of particular value in the present invention as a delivery vector.

[0444] 2. Retroviruses

[0445] The retroviruses are a group of single-stranded RNA viruses characterised by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription (Coffin, 1990). The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes, gag, pol, and env that code for capsid proteins, polymerase enzyme, and envelope components, respectively. A sequence found upstream from the gag gene contains a signal for packaging of the genome into virions. Two long terminal repeat (LTR) sequences are present at the 5' and 3' ends of the viral genome. These contain strong promoter and enhancer sequences and are also required for integration in the host cell genome (Coffin, 1990).

[0446] In order to construct a retroviral vector, a nucleic acid encoding one or more oligonucleotide or polynucleotide sequences of interest is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line containing the gag, pol, and env genes but without the LTR and packaging components is constructed (Mann et al., 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into this cell line (by calcium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas & Rubenstein, 1988; Temin, 1986; Mann et al., 1983). The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of

cell types. However, integration and stable expression require the division of host cells (Paskind et al., 1975).

[0447] A novel approach designed to allow specific targeting of retrovirus vectors was recently developed based on the chemical modification of a retrovirus by the chemical addition of lactose residues to the viral envelope. This modification could permit the specific infection of hepatocytes via sialoglycoprotein receptors.

[0448] A different approach to targeting of recombinant retroviruses was designed in which biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor were used. The antibodies were coupled via the biotin components by using streptavidin (Roux et al., 1989). Using antibodies against major histocompatibility complex class I and class II antigens, they demonstrated the infection of a variety of human cells that bore those surface antigens with an ecotropic virus *in vitro* (Roux et al., 1989).

[0449] 3. Adeno-Associated Viruses

[0450] AAV (Ridgeway, 1988; Hermonat & Muzyczka, 1984) is a parovirus, discovered as a contamination of adenoviral stocks. It is a ubiquitous virus (antibodies are present in 85% of the US human population) that has not been linked to any disease. It is also classified as a dependovirus, because its replication is dependent on the presence of a helper virus, such as adenovirus. Five serotypes have been isolated, of which AAV-2 is the best characterised. AAV has a single-stranded linear DNA that is encapsidated into capsid proteins VP1, VP2 and VP3 to form an icosahedral virion of 20 to 24 nm in diameter (Muzyczka & McLaughlin, 1988).

[0451] The AAV DNA is approximately 4.7 kilobases long. It contains two open reading frames and is flanked by two ITRs. There are two major genes in the AAV genome: rep and cap. The rep gene codes for proteins responsible for viral replications, whereas cap codes for capsid protein VP1-3. Each ITR forms a T-shaped hairpin structure. These terminal repeats are the only essential cis components of the AAV for chromosomal integration. Therefore, the AAV can be used as a vector with all viral coding sequences removed and replaced by the cassette of genes for delivery. Three viral promoters have been identified and named p5, p19, and p40, according to their map position. Transcription from p5 and p19 results in production of rep proteins, and transcription from p40 produces the capsid proteins (Hermonat & Muzyczka, 1984).

[0452] There are several factors that prompted researchers to study the possibility of using rAAV as an expression vector. One is that the requirements for delivering a gene to integrate into the host chromosome are surprisingly few. It is necessary to have the 145-bp ITRs, which are only 6% of the AAV genome. This leaves room in the vector to assemble a 4.5-kb DNA insertion. While this carrying capacity may prevent the AAV from delivering large genes, it is amply suited for delivering antisense constructs.

[0453] AAV is also a good choice of delivery vehicles due to its safety. There is a relatively complicated rescue mechanism: not only wild type adenovirus but also AAV genes are required to mobilise rAAV. Likewise, AAV is not pathogenic and not associated with any disease. The removal of viral coding sequences minimises immune reactions to viral gene expression, and therefore, rAAV does not evoke an inflammatory response.

[0454] 4. Other Viral Vectors as Expression Constructs

[0455] Other viral vectors may be employed as expression constructs in the present invention for the delivery of oligonucleotide or polynucleotide sequences to a host cell. Vectors

derived from viruses such as vaccinia virus (Ridgeway, 1988; Coupar et al., 1988), lentiviruses, polio viruses and herpes viruses may be employed. Other poxvirus derived vectors, such as fowl-pox derived vectors, may also be expected to be of use. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Coupar et al., 1988; Horwich et al., 1990).

[0456] With the recent recognition of defective hepatitis B viruses, new insight was gained into the structure-function relationship of different viral sequences. *In vitro* studies showed that the virus could retain the ability for helper-dependent packaging and reverse transcription despite the deletion of up to 80% of its genome (Horwich et al., 1990). This suggested that large portions of the genome could be replaced with foreign genetic material. The hepatotropism and persistence (integration) were particularly attractive properties for liver-directed gene transfer. Chang et al. (1991) introduced the chloramphenicol acetyltransferase (CAT) gene into duck hepatitis B virus genome in the place of the polymerase, surface, and pre-surface coding sequences. It was cotransfected with wild-type virus into an avian hepatoma cell line. Culture media containing high titres of the recombinant virus were used to infect primary duckling hepatocytes. Stable CAT gene expression was detected for at least 24 days after transfection (Chang et al., 1991).

[0457] Additional "viral" vectors include virus like particles (VLPs) and phages.

[0458] 5. Non-Viral Vectors

[0459] In order to effect expression of the oligonucleotide or polynucleotide sequences of the present invention, the expression construct must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cells lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states. As described above, one preferred mechanism for delivery is via viral infection where the expression construct is encapsulated in an infectious viral particle.

[0460] Once the expression construct has been delivered into the cell the nucleic acid encoding the desired oligonucleotide or polynucleotide sequences may be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the construct may be stably integrated into the genome of the cell. This integration may be in the specific location and orientation via homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronisation with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

[0461] In certain embodiments of the invention, the expression construct comprising one or more oligonucleotide or polynucleotide sequences may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed, for example, by any method which physically or chemically permeabilises the cell membrane. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* use as well. Dubensky et al. (1984) successfully injected polyomavirus DNA in the form of calcium phosphate precipitates into liver and spleen of adult and newborn mice

demonstrating active viral replication and acute infection. Benvenisty & Reshef (1986) also demonstrated that direct intraperitoneal injection of calcium phosphate-precipitated plasmids results in expression of the transfected genes. It is envisioned that DNA encoding a gene of interest may also be transferred in a similar manner in vivo and express the gene product.

[0462] Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA-coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein et al., 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang et al., 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

[0463] Selected organs including the liver, skin, and muscle tissue of rats and mice have been bombarded in vivo (Yang et al., 1990; Zelenin et al., 1991). This may require surgical exposure of the tissue or cells, to eliminate any intervening tissue between the gun and the target organ, i.e., ex vivo treatment. Again, DNA encoding a particular gene may be delivered via this method and still be incorporated.

[0464] Bacteria may also be utilised as a delivery method (e.g. *listeria*, see WO2004/11048) and in particular BCG.

Polypeptide Compositions

[0465] The present invention, in other aspects, provides polypeptide compositions.

[0466] Generally, a polypeptide of the invention will be an isolated polypeptide (i.e. separated from those components with which it may usually be found in nature).

[0467] For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

[0468] Polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described above may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, and higher eukaryotic cells, such as mammalian cells and plant cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

[0469] Polypeptides of the invention, immunogenic fragments thereof, and other variants having less than about 100

amino acids, and generally less than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesised using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, *J. Am. Chem. Soc.* 85:2149-2146 (1963). Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied Bio-Systems Division (Foster City, Calif.), and may be operated according to the manufacturer's instructions.

[0470] Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, examples of such proteins include tetanus, tuberculosis and hepatitis proteins (see, e.g., Stoute et al., *New Engl. J. Med.* 336:86-91 (1997)). A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognised by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein.

[0471] Fusion proteins may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion protein is expressed as a recombinant protein, allowing the production of increased levels, relative to a non-fused protein, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion protein that retains the biological activity of both component polypeptides.

[0472] A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene* 40:39-46 (1985); Murphy et al., *Proc. Natl. Acad. Sci. USA* 83:8258-8262 (1986); U.S. Pat. No. 4,935,233 and U.S. Pat. No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required

when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

[0473] Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium *Haemophilus influenza* B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (e.g., the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemagglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

[0474] In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the *Lyta* gene; *Gene* 43:265-292 (1986)). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (see *Biotechnology* 10:795-798 (1992)). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

T Cells

[0475] Immunotherapeutic compositions may also, or alternatively, comprise T cells specific for a *Mycobacterium* antigen. Such cells may generally be prepared in vitro or ex vivo, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood, or a fraction of bone marrow or peripheral blood of a patient, using a commercially available cell separation system, such as the Isolex™ System, available from Nexell Therapeutics, Inc. (Irvine, Calif.; see also U.S. Pat. No. 5,240,856; U.S. Pat. No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

[0476] T cells may be stimulated with a polypeptide of the invention, polynucleotide encoding such a polypeptide, and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide. Preferably, the polypeptide or polynucleotide is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.

[0477] T cells are considered to be specific for a polypeptide of the invention if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the polypep-

tide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen et al., *Cancer Res.* 54:1065-1070 (1994)). Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (e.g., by pulse-labelling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a polypeptide of the invention (100 ng/ml-100 µg/ml, preferably 200 ng/ml-25 µg/ml) for 3-7 days should result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (e.g., TNF or IFN-γ) is indicative of T cell activation (see Coligan et al., *Current Protocols in Immunology*, vol. 1 (1998)). T cells that have been activated in response to a polypeptide, polynucleotide or polypeptide-expressing APC may be CD4⁺ and/or CD8⁺. Protein-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T cells are derived from a patient, a related donor or an unrelated donor, and are administered to the patient following stimulation and expansion.

[0478] For therapeutic purposes, CD4⁺ or CD8⁺ T cells that proliferate in response to a polypeptide, polynucleotide or APC can be expanded in number either in vitro or in vivo. Proliferation of such T cells in vitro may be accomplished in a variety of ways. For example, the T cells can be re-exposed to a polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesise a polypeptide. Alternatively, one or more T cells that proliferate in the presence of the protein can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

pharmaceutical compositions

[0479] In additional embodiments, the polynucleotide, polypeptide, T-cell and/or antibody compositions disclosed herein will be formulated in pharmaceutically-acceptable or physiologically-acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy.

[0480] It will also be understood that, if desired, the nucleic acid segment (e.g., RNA or DNA) that expresses a polypeptide as disclosed herein may be administered in combination with other agents as well, such as, e.g., other proteins or polypeptides or various pharmaceutically-active agents, including chemotherapeutic agents effective against a *M. tuberculosis* infection. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The compositions may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesised as described

herein. Likewise, such compositions may further comprise substituted or derivatised RNA or DNA compositions.

[0481] Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation. Other routes of administration include via the mucosal surfaces.

[0482] Typically, formulations comprising a therapeutically effective amount deliver about 0.1 ug to about 1000 ug of polypeptide per administration, more typically about 2.5 ug to about 100 ug of polypeptide per administration. In respect of polynucleotide compositions, these typically deliver about 10 ug to about 20 mg of the inventive polynucleotide per administration, more typically about 0.1 mg to about 10 mg of the inventive polynucleotide per administration.

[0483] Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared in such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

[0484] 1. Oral Delivery

[0485] In certain applications, the pharmaceutical compositions disclosed herein may be delivered via oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

[0486] The active compounds may even be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (Mathiowitz et al., 1997; Hwang et al., 1998; U.S. Pat. No. 5,641,515; U.S. Pat. No. 5,580,579 and U.S. Pat. No. 5,792,451, each specifically incorporated herein by reference in its entirety). The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavouring agent, such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. A syrup or elixir may contain the active component, sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavouring, such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active components may be incorporated into sustained-release preparation and formulations.

[0487] For oral administration the compositions of the present invention may alternatively be incorporated with one

or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally-administered formulation. For example, a mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically-effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

[0488] 2. Injectable Delivery

[0489] In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein parenterally, intravenously, intramuscularly, intradermally, or even intraperitoneally as described in U.S. Pat. No. 5,543,158; U.S. Pat. No. 5,641,515 and U.S. Pat. No. 5,399,363 (each specifically incorporated herein by reference in its entirety). Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0490] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Pat. No. 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0491] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of

hypodermoclysis fluid or injected at the proposed site of infusion (see, e.g., *Remington's Pharmaceutical Sciences*, 15th Edition, pp. 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

[0492] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0493] The compositions disclosed herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

[0494] As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

[0495] The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

[0496] 3. Nasal and Buccal Delivery

[0497] In certain embodiments, the pharmaceutical compositions may be delivered by intranasal sprays, buccal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, nucleic acids, and peptide compositions directly to the lungs eg via nasal and buccal

aerosol sprays has been described e.g., in U.S. Pat. No. 5,756,353 and U.S. Pat. No. 5,804,212 (each specifically incorporated herein by reference in its entirety). Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga et al., 1998) and lysophosphatidyl-glycerol compounds (U.S. Pat. No. 5,725,871, specifically incorporated herein by reference in its entirety) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroethylene support matrix is described in U.S. Pat. No. 5,780,045 (specifically incorporated herein by reference in its entirety).

[0498] 4. Liposome-, Nanocapsule-, and Microparticle-Mediated Delivery

[0499] In certain embodiments, the inventors contemplate the use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present invention into suitable host cells. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

[0500] Such formulations may be preferred for the introduction of pharmaceutically-acceptable formulations of the nucleic acids or constructs disclosed herein. The formation and use of liposomes is generally known to those of skill in the art (see for example, Couvreur et al., 1977; Couvreur, 1988; Lasic, 1998; which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy for intracellular bacterial infections and diseases). Recently, liposomes were developed with improved serum stability and circulation half-times (Gabizon & Papahadjopoulos, 1988; Allen and Choun, 1987; U.S. Pat. No. 5,741,516, specifically incorporated herein by reference in its entirety). Further, various methods of liposome and liposome like preparations as potential drug carriers have been reviewed (Takakura, 1998; Chandran et al., 1997; Margalit, 1995; U.S. Pat. No. 5,567,434; U.S. Pat. No. 5,552,157; U.S. Pat. No. 5,565,213; U.S. Pat. No. 5,738,868 and U.S. Pat. No. 5,795,587, each specifically incorporated herein by reference in its entirety).

[0501] Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Renneisen et al., 1990; Muller et al., 1990). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs (Heath & Martin, 1986; Heath et al., 1986; Balazsovits et al., 1989; Fresta & Puglisi, 1996), radiotherapeutic agents (Pikul et al., 1987), enzymes (Imaizumi et al., 1990a; Imaizumi et al., 1990b), viruses (Faller & Baltimore, 1984), transcription factors and allosteric effectors (Nicolau & Gersonde, 1979) into a variety of cultured cell lines and animals. In addition, several successful clinical trials examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein et al., 1985a; 1985b; Coune, 1988; Sculier et al., 1988). Furthermore, several studies suggest that the use of liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mon & Fukatsu, 1992).

[0502] Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 μ m. Sonication of MLVs results in the

formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

[0503] Liposomes bear resemblance to cellular membranes and are contemplated for use in connection with the present invention as carriers for the peptide compositions. They are widely suitable as both water- and lipid-soluble substances can be entrapped, i.e. in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

[0504] In addition to the teachings of Couvreur et al. (1977; 1988), the following information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

[0505] In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins, such as cytochrome c, bind, deform and penetrate the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

[0506] The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution, however, and a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUVs). These are prepared by ether evaporation and are three to four times more efficient at solute entrapment than MLVs.

[0507] In addition to liposome characteristics, an important determinant in entrapping compounds is the physicochemical properties of the compound itself. Polar compounds are trapped in the aqueous spaces and nonpolar compounds bind to the lipid bilayer of the vesicle. Polar compounds are released through permeation or when the bilayer is broken, but nonpolar compounds remain affiliated with the bilayer unless it is disrupted by temperature or exposure to lipoproteins. Both types show maximum efflux rates at the phase transition temperature.

[0508] Liposomes interact with cells via four different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of

liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. It often is difficult to determine which mechanism is operative and more than one may operate at the same time.

[0509] The fate and disposition of intravenously injected liposomes depend on their physical properties, such as size, fluidity, and surface charge. They may persist in tissues for hours or days, depending on their composition, and half lives in the blood range from minutes to several hours. Larger liposomes, such as MLVs and LUVs, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but physiology of the circulatory system restrains the exit of such large species at most sites. They can exit only in places where large openings or pores exist in the capillary endothelium, such as the sinusoids of the liver or spleen. Thus, these organs are the predominate site of uptake. On the other hand, SUVs show a broader tissue distribution but still are sequestered highly in the liver and spleen. In general, this *in vivo* behaviour limits the potential targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow, and lymphoid organs.

[0510] Targeting is generally not a limitation in terms of the present invention. However, should specific targeting be desired, methods are available for this to be accomplished. Antibodies may be used to bind to the liposome surface and to direct the antibody and its drug contents to specific antigenic receptors located on a particular cell-type surface. Carbohydrate determinants (glycoprotein or glycolipid cell-surface components that play a role in cell-cell recognition, interaction and adhesion) may also be used as recognition sites as they have potential in directing liposomes to particular cell types. Mostly, it is contemplated that intravenous injection of liposomal preparations would be used, but other routes of administration are also conceivable.

[0511] Alternatively, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland et al., 1987; Quintanar-Guerrero et al., 1998; Douglas et al., 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 µm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention. Such particles may be easily made, as described (Couvreur et al., 1980; 1988; zur Muhlen et al., 1998; Zambaux et al. 1998; Pinto-Alphandry et al., 1995 and U.S. Pat. No. 5,145,684, specifically incorporated herein by reference in its entirety).

[0512] Skin patches may also be utilised for transcutaneous delivery.

Immunogenic Compositions

[0513] In certain preferred embodiments of the present invention, immunogenic compositions are provided. The immunogenic compositions will generally comprise one or more polypeptides or polynucleotides, such as those discussed above, in combination with an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (e.g.,

polylactic galactide) and liposomes (into which the compound is incorporated; see, e.g., Fullerton, U.S. Pat. No. 4,235,877).

[0514] Preparation of Immunogenic Compositions is Generally Described in, for Example, Powell & Newman, eds., *Vaccine Design* (the subunit and adjuvant approach) (1995). Pharmaceutical compositions and immunogenic compositions within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other *M. tuberculosis* antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the pharmaceutical or immunogenic composition.

[0515] Illustrative immunogenic compositions may contain a polynucleotide (e.g. DNA) encoding one or more of the polypeptides as described above, such that the polypeptide is generated in situ (thereby eliciting an immune response). As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems* 15:143-198 (1998), and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium host cell (for example, a *Mycobacterium*, *Bacillus* or *Lactobacillus* strain, including *Bacillus-Calmette-Guerrin* or *Lactococcus lactis*) that expresses the polypeptide (e.g. on its cell surface or secretes the polypeptide) (see, for example, Ferreira, et al., *An Acad Bras Cienc* (2005) 77:113-124; and Raha, et al., *Appl Microbiol Biotechnol* (2005) PubMedID 15635459). In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch et al., *Proc. Natl. Acad. Sci. USA* 86:317-321 (1989); Flexner et al., *Ann. N.Y. Acad. Sci.* 569:86-103 (1989); Flexner et al., *Vaccine* 8:17-21 (1990); U.S. Pat. Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Pat. No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques* 6:616-627 (1988); Rosenfeld et al., *Science* 252:431-434 (1991); Kolls et al., *Proc. Natl. Acad. Sci. USA* 91:215-219 (1994); Kass-Eisler et al., *Proc. Natl. Acad. Sci. USA* 90:11498-11502 (1993); Guzman et al., *Circulation* 88:2838-2848 (1993); and Guzman et al., *Cir. Res.* 73:1202-1207 (1993). Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science* 259:1745-1749 (1993) and reviewed by Cohen, *Science* 259:1691-1692 (1993). The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells. It will be apparent that a immunogenic composition may comprise both a polynucleotide and a polypeptide component. Such immunogenic composition may provide for an enhanced immune response. It will be apparent that an immunogenic composition may contain pharmaceutically acceptable salts of the polynucleotides and polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (e.g.,

salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (e.g., sodium, potassium, lithium, ammonium, calcium and magnesium salts).

[0516] While any suitable carrier known to those of ordinary skill in the art may be employed in the immunogenic compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Pat. Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344 and 5,942,252. One may also employ a carrier comprising the particulate-protein complexes described in U.S. Pat. No. 5,928,647, which are capable of inducing a class I-restricted cytotoxic T lymphocyte responses in a host.

[0517] Such compositions may also comprise buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

[0518] Any of a variety of immunostimulants may be employed in the immunogenic compositions of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminium hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *Mycobacterium* species or *Mycobacterium* derived proteins. For example, delipidated, deglycolipidated *M. vaccae* ("pVac") can be used. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Mich.); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.); AS01B, AS02A, AS15, AS-2 and derivatives thereof (GlaxoSmithKline, Philadelphia, Pa.); CWS (cell wall skeleton from a tubercule *bacillus*), TDM (trehalose dicorynomycolate), Leif (Leishmania elongation initiation factor), aluminium salts such as aluminium hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A (MPL®); and quil A (e.g. QS-21). Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

[0519] An adjuvant refers to the components in a vaccine or therapeutic composition that increase the specific immune response to the antigen (see, e.g., Edelman, *AIDS Res. Hum Retroviruses* 8:1409-1411 (1992)). Adjuvants induce immune responses of the Th1-type and Th-2 type response. Th1-type cytokines (e.g., IFN- γ , IL-2, and IL-12) tend to favour the induction of cell-mediated immune response to an administered antigen, while Th-2 type cytokines (e.g., IL-4, IL-5, IL-6, IL-10) tend to favour the induction of humoral immune responses. Adjuvants capable of preferential stimulation of a Th-1 cell-mediated immune response are described in WO 94/00153 and WO 95/17209.

[0520] Within the immunogenic compositions provided herein, the adjuvant composition is preferably designed to induce an immune response predominantly of the Th1 type. Following application of an immunogenic composition as provided herein, a patient will typically support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Janeway, et al., *Immunobiology*, 5th Edition, 2001.

[0521] The Rv2386c compositions usually comprise one or more adjuvants, e.g., AS01B (3-de-O-acylated monophosphoryl lipid A (3D-MPL®) and QS21 in a liposome formulation; see, U.S. Patent Publication No. 2003/0143240); AS02A (3D-MPL® and QS21 and an oil in water emulsion; see, Bojang, et al., *Lancet* (2001) 358:1927); ENHANZYN® (Detox); 3D-MPL®; saponins including Quil A and its components e.g. QS21 and saponin mimetics; CWS (cell wall skeleton from a tubercule *bacillus*); TDM (trehalose dicorynomycolate); aminoalkyl glucosaminide 4-phosphates (AGPs); immunostimulatory oligonucleotides e.g. CPG; Leif (Leishmania elongation initiation factor); and derivatives thereof. In a preferred embodiment, an Rv2386c polypeptide is administered with one or more adjuvants selected from the group consisting of 3D-MPL® and QS21 in a liposome formulation eg AS01B and 3D-MPL® and QS21 and an oil in water emulsion (e.g. AS02A). Adjuvant systems AS01B and AS02A are further described in Pichyangkul, et al., *Vaccine* (2004) 22:3831-40.

[0522] When delivering the Rv2386c antigen as a nucleic acid, it can be delivered, for example, in a viral vector (i.e., an adenovirus vector), or in a mutant bacterium host cell (i.e., a mutant, avirulent *Mycobacterium*, *Lactobacillus* or *Bacillus* host cell including *Bacillus Calmette-Guerin* (BCG) and *Lactococcus lactis*).

[0523] Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A (MPL®), preferably 3-O-deacylated monophosphoryl lipid A (3D-MPL®), optionally with an aluminium salt (see, for example, Ribí, et al., 1986, *Immunology and Immunopharmacology of Bacterial Endotoxins*, Plenum Publ. Corp., NY, pp. 407-419; GB 2122204B; GB 2220211; and U.S. Pat. No. 4,912,094). A preferred form of 3D-MPL® is in the form of an emulsion having a small particle size less than 0.2 μ m in diameter, and its method of manufacture is disclosed in WO 94/21292. Aqueous formulations comprising monophosphoryl lipid A and a surfactant have been described in WO 98/43670. Exemplified preferred adjuvants include AS01B (MPL® and QS21 in a liposome formulation), 3D-MPL® and QS21 in a liposome formula-

tion, AS02A (MPL® and QS21 and an oil in water emulsion), 3D-MPL® and QS21 and an oil in water emulsion, and AS15, available from GlaxoSmithKline. MPL® adjuvants are available from GlaxoSmithKline (see U.S. Pat. Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094).

[0524] CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. CpG is an abbreviation for cytosine-guanosine dinucleotide motifs present in DNA. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Pat. Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato et al., *Science* 273:352 (1996). CpG when formulated into immunogenic compositions, is generally administered in free solution together with free antigen (WO 96/02555; McCluskie and Davis, supra) or covalently conjugated to an antigen (WO 98/16247), or formulated with a carrier such as aluminium hydroxide ((Hepatitis surface antigen) Davis et al. supra; Brazolot-Millan et al., *Proc. Natl. Acad. Sci., USA*, 1998, 95(26), 15553-8). CpG is known in the art as being an adjuvant that can be administered by both systemic and mucosal routes (WO 96/02555, EP 468520, Davis et al., *J. Immunol.*, 1998, 160(2):870-876; McCluskie and Davis, *J. Immunol.*, 1998, 161(9):4463-6).

[0525] Another preferred adjuvant is a saponin or saponin mimetics or derivatives, such as Quil A, preferably QS21 (Aquila Biopharmaceuticals Inc., Framingham, Mass.), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A (MPL®) and saponin derivative, such as the combination of QS21 and 3D-MPL® as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL® and tocopherol in an oil-in-water emulsion is described in WO 95/17210. Additional saponin adjuvants of use in the present invention include QS7 (described in WO 96/33739 and WO 96/11711) and QS17 (described in U.S. Pat. No. 5,057,540 and EP 0 362 279 B1).

[0526] Alternatively the saponin formulations may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, poly-N-acetyl glucosamine-based polymer matrix, particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, etc. The saponins may also be formulated in the presence of cholesterol to form particulate structures such as liposomes or ISCOM®s. Furthermore, the saponins may be formulated together with a polyoxyethylene ether or ester, in either a non-particulate solution or suspension, or in a particulate structure such as a paucilamellar liposome or ISCOM®. The saponins may also be formulated with excipients such as CARBOPOL® to increase viscosity, or may be formulated in a dry powder form with a powder excipient such as lactose.

[0527] In one embodiment, the adjuvant system includes the combination of a monophosphoryl lipid A and a saponin derivative, such as the combination of QS21 and 3D-MPL® adjuvant, as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol containing liposomes, as described in WO 96/33739. Other suitable formulations comprise an oil-in-water emulsion and

tocopherol. Another suitable adjuvant formulation employing QS21, 3D-MPL® adjuvant and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

[0528] Another enhanced adjuvant system involves the combination of a CpG-containing oligonucleotide and a saponin derivative particularly the combination of CpG and QS21 as disclosed in WO 00/09159. Suitably the formulation additionally comprises an oil in water emulsion and tocopherol.

[0529] Other suitable adjuvants include MONTANIDE® ISA 720 (Seppic, France), SAF (Chiron, Calif., United States), ISCOMS® (CSL), MF-59 (Chiron), the SBAS series of adjuvants (SmithKline Beecham, Rixensart, Belgium), Detox (Corixa), RC-529 (Corixa) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. patent application Ser. Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and polyoxyethylene ether adjuvants such as those described in WO 99/52549A1. SmithKline Beecham and Corixa Corporation are now part of GlaxoSmithKline.

[0530] Other suitable adjuvants include adjuvant molecules of the general formula (I):



wherein, n is 1-50, A is a bond or —C(O)—, R is C₁₋₅₀ alkyl or Phenyl C₁₋₅₀ alkyl.

[0531] A further adjuvant of interest is shiga toxin b chain, used for example as described in WO2005/112991.

[0532] One embodiment of the present invention consists of an immunogenic composition comprising a polyoxyethylene ether of general formula (I), wherein n is between 1 and 50, preferably 4-24, most preferably 9; the R component is C₁₋₅₀, preferably C₄-C₂₀ alkyl and most preferably C₁₋₂ alkyl, and A is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12th edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

[0533] Any immunogenic composition provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained release formulation (i.e., a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (see, e.g., Coombes et al., *Vaccine* 14:1429-1438 (1996)) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

[0534] Carriers for use within such formulations are bio-compatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release.

[0535] Such carriers include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (e.g., a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (see, e.g., U.S. Pat. No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release.

[0536] Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and immunogenic compositions to facilitate production of an antigen-specific immune response. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response and/or to be immunologically compatible with the receiver (i.e., matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

[0537] Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau & Steinman, *Nature* 392:245-251 (1998)) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic immunity (see Timmerman & Levy, *Ann. Rev. Med.* 50:507-529 (1999)). In general, dendritic cells may be identified based on their typical shape (stellate in situ, with marked cytoplasmic processes (dendrites) visible in vitro), their ability to take up, process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells in vivo or ex vivo, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within an immunogenic composition (see Zitvogel et al., *Nature Med.* 4:594-600 (1998)).

[0538] Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated ex vivo by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

[0539] Dendritic cells are conveniently categorised as “immature” and “mature” cells, which allows a simple way to

discriminate between two well characterised phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterised as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcγ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1BB).

[0540] APCs may generally be transfected with a polynucleotide encoding a protein (or portion or other variant thereof) such that the polypeptide, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a pharmaceutical composition or immunogenic composition comprising such transfected cells may then be used, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., *Immunology and Cell Biology* 75:456-460 (1997). Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

[0541] Immunogenic compositions and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are preferably hermetically sealed to preserve sterility of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, an immunogenic composition or pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

[0542] In some embodiments, a “priming” or first administration of an Rv2386c polypeptide (including variants, immunogenic fragments or fusion proteins), or polynucleotide encoding said polypeptide, is followed by one or more “boosting” or subsequent administrations of an Rv2386c polypeptide (including variants, immunogenic fragments or fusion proteins) or polynucleotide encoding said polypeptide (“prime and boost” method). For instance, a first administration with an Rv2386c polypeptide (including variants, immunogenic fragments or fusion proteins) or polynucleotide encoding said polypeptide is followed by one or more subsequent administrations of an Rv2386c polypeptide (including variants, immunogenic fragments or fusion proteins) or polynucleotide encoding said polypeptide.

[0543] In one embodiment, a first administration with an Rv2386c polypeptide or polynucleotide is followed by one or more subsequent administrations of an Rv2386c polypeptide. In one embodiment, a first administration with an Rv2386c

polypeptide or polynucleotide is followed by one or more subsequent administrations of an Rv2386c polynucleotide. Usually the first or “priming” administration and the second or “boosting” administration are given about 2-12 weeks apart, or up to 4-6 months apart. Subsequent “booster” administrations are given about 6 months apart, or as long as 1, 2, 3, 4 or 5 years apart. Conventional booster treatment (e.g., a protein priming administration followed by a protein boosting administration) may also be useful in preventing or treating tuberculosis (e.g. preventing or treating latent tuberculosis, in particular preventing or delay tuberculosis reactivation).

Antibodies

[0544] “Antibody” refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognises an antigen. The recognised immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[0545] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kDa) and one “heavy” chain (about 50-70 kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0546] Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterised fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The $F(ab)_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (see *Fundamental Immunology* (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesised *de novo* using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (see, e.g., McCafferty et al., *Nature* 348:552-554 (1990)).

[0547] For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used (see, e.g., Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor et al., *Immunology Today* 4: 72 (1983); Cole et al., pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy* (1985)). Techniques for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanised antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab

fragments that specifically bind to selected antigens (see, e.g., McCafferty et al., *Nature* 348:552-554 (1990); Marks et al., *Biotechnology* 10:779-783 (1992)).

[0548] The phrase “specifically (or selectively) binds” to an antibody or “specifically (or selectively) immunoreactive with,” when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised to fusion proteins can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with fusion protein and not with individual components of the fusion proteins. This selection may be achieved by subtracting out antibodies that cross-react with the individual antigens. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, *Antibodies, A Laboratory Manual* (1988) and *Using Antibodies: A Laboratory Manual* (1998), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10, 20 or 100 times background (e.g. binding to other *Mycobacterium* proteins, such as other *Mycobacterium tuberculosis* proteins).

Diagnostics

[0549] In another aspect, this invention provides methods for using one or more of the polypeptides described above to diagnose tuberculosis (for example using T cell response based assays or antibody based assays of conventional format).

[0550] For example, there is provided a method for determining prior *M. tuberculosis* infection in an individual comprising:

[0551] (a) obtaining a sample from the individual;

[0552] (b) contacting said sample with an isolated polypeptide which comprises:

[0553] (i) an Rv2386c protein sequence;

[0554] (ii) a variant of an Rv2386c protein sequence; or

[0555] (iii) an immunogenic fragment of an Rv2386c protein sequence;

[0556] (c) quantifying the sample response.

[0557] The sample may for example be whole blood or purified cells. Suitably the sample will contain peripheral blood mononucleated cells (PBMC). In one embodiment of the invention the individual will be seropositive. In a second embodiment of the invention the individual will be seronegative.

[0558] Suitably the individual will not previously have been vaccinated against *M. tuberculosis* infection (e.g. suitably the individual will not previously have been vaccinated with BCG).

[0559] The sample response may be quantified by a range of means known to those skilled in the art, including the

monitoring of lymphocyte proliferation or the production of specific cytokines or antibodies. For example, T-cell ELISPOT may be used to monitor cytokines such as interferon gamma (IFN γ), interleukin 2 (IL2) and interleukin 5 (IL5). B-cell ELISPOT may be used to monitor the stimulation of *M. tuberculosis* specific antigens. The cellular response may also be characterised by the use of by intra- and extra-cellular staining and analysis by a flow cytometer.

[0560] Methods of quantifying a sample proliferation response include:

[0561] (i) pulsing cultured cells with a radiolabel (e.g. tritiated thymidine) and monitoring tritium uptake (e.g. gas scintillation);

[0562] (ii) carboxyfluorescein diacetate succinimidyl ester (CFSE) labelling and fluorescence monitoring of cell division using flow cytometry.

[0563] Quantifying a sample cytokine response includes in particular the monitoring of interferon gamma production.

[0564] When using such quantification methods, a positive response to an antigen may be defined by a signal to noise ratio (S/N ratio) of at least 2:1 (for example, at least 3:1 or at least 5:1).

[0565] In a further aspect of the present invention methods are provided to diagnose *M. tuberculosis* infection using a skin test. As used herein, a “skin test” is any assay performed directly on a patient in which a delayed-type hypersensitivity (DTH) reaction (such as swelling, reddening or dermatitis) is measured following intradermal injection of an Rv153c polypeptide as described above (or variant, immunogenic fragments thereof or nucleotides encoding them). Such injection may be achieved using any suitable device sufficient to contact the antigen combinations with dermal cells of the patient, such as a tuberculin syringe or 1 mL syringe. The reaction is measured after a period of time, for example at least 48 hours after injection, especially 48-72 hours.

[0566] The DTH reaction is a cell-mediated immune response, which is greater in patients that have been exposed previously to the test antigen. The response may be measured visually, using a ruler. In general, a response that is greater than about 0.5 cm in diameter, especially greater than about 1.0 cm in diameter, is a positive response, indicative of prior *M. tuberculosis* infection, which may or may not be manifested as an active disease.

[0567] For use in a skin test, the Rv2386c component is suitably formulated as a pharmaceutical composition containing a physiologically acceptable carrier. Suitably, the carrier employed in such pharmaceutical compositions is a saline solution with appropriate preservatives, such as phenol and/or Tween 80TM.

[0568] The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment.

[0569] For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically binds to a protein. Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

[0570] Alternatively, a kit may be designed to detect the level of mRNA encoding a protein in a biological sample. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridises to a polynucleotide encoding a protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding a protein of the invention.

[0571] Other diagnostics kits include those designed for the detection of cell mediated responses (which may, for example, be of use in the diagnostic methods of the present invention). Such kits will typically comprise:

[0572] (i) apparatus for obtaining an appropriate cell sample from a subject;

[0573] (ii) means for stimulating said cell sample with an Rv2386c polypeptide (or variant thereof, immunogenic fragments thereof, or DNA encoding such polypeptides);

[0574] (iii) means for detecting or quantifying the cellular response to stimulation.

[0575] Suitable means for quantifying the cellular response include a B-cell ELISPOT kit or alternatively a T-cell ELISPOT kit, which are known to those skilled in the art.

[0576] One possible kit comprises:

[0577] (a) a polypeptide of the invention; and

[0578] (b) a detection reagent suitable for direct or indirect detection of antibody binding.

[0579] Of particular interest are diagnostic kits tailored for quantifying T cell responses:

[0580] A diagnostic kit comprising:

[0581] (a) a polypeptide of the invention; and

[0582] (b) apparatus sufficient to contact said polypeptide with the dermal cells of an individual.

[0583] A diagnostic kit comprising:

[0584] (a) a polypeptide of the invention;

[0585] (b) apparatus sufficient to contact said polypeptide with a sample (e.g. whole blood or more suitably PBMC) from an individual; and

[0586] (c) means to quantify the T cell response (e.g. proliferation or IFN-gamma production).

EXAMPLES

[0587] The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

Example 1

Identification of Rv2386c as a Latent TB Vaccine Target

[0588] The gene Rv2386c also known as MbtI, encodes for a protein which is involved in the biogenesis of hydroxyphenyloxazoline-containing siderophore mycobactins where it converts chorismate to salicylate, the starter unit for mycobacterin siderophore synthesis.

[0589] Rv2386c was selected based on a genome-wide analysis of *Mycobacterium tuberculosis* genes associated with dormancy phase maintenance and infectivity as in Murphy and Brown *BMC. Infect. Dis.* 2007 7:84-99. Potential dormancy phase gene targets in *Mycobacterium tuberculosis* were prioritised through a bioinformatics meta-analysis of published genome-wide DNA microarray datasets of bacterial gene expression under simulated dormancy conditions. Subcellular localisation of *M. tuberculosis* proteins encoded by genes, was subsequently carried out on the entire genome to identify vaccine targets.

[0590] Briefly, experimental conditions in the dormancy models were quite varied so a zero to five scoring system was developed to normalise these data based upon two criteria: 1) the relevance of the experimental conditions to the dormant state and 2) the rank order of expression. The maximum score for a particular experimental dataset was adjusted based on potential relevance to the clinical occurrence of dormancy phase *M. tuberculosis* infections. Table 1 shows the data sets collected for Step 1 together with the adjusted maximum scores for each dataset. Additional datasets on gene essentiality for growth were obtained from published studies using transposon-based knockout experiments (TraSH). Genes which had no effect on growth received a score of zero.

TABLE 1

Sources, experimental models, and scoring criteria for <i>M. tuberculosis</i> DNA microarray gene expression and genome-wide gene knock-out (growth phase essentiality).		
Reference	Experimental model	Timepoint: Maximum score ^a
Betts JC et al. <i>Mol. Microbiol.</i> 2002 43: 717-731	Starvation under controlled O ₂	96 h: 3 24 h: 2 4 h: 1
Hampshire T et al. <i>Tuberculosis.(Edinb.)</i> 2004 84: 228-238	Nutrient depletion under controlled O ₂	62 and 75 d: 5 49 d: 4 18 d: 2
Muttucumaru DG et al. <i>Tuberculosis.(Edinb.)</i> 2004 84: 239-246	Wayne model of hypoxia [#]	14 d (NRP-2): 4 7 d (NRP-1): 2
Voskuil MI et al. <i>Tuberculosis.(Edinb.)</i> 2004 84: 218-227	Wayne model of hypoxia [#]	30 and 80 d: 5 14 and 20 d: 4 10 and 12 d: 3 6 and 8 d: 2
Schnappinger D et al. <i>J. Exp. Med.</i> 2003 198: 693-704	Infection of mouse macrophages, +/- γ-INF	24 and 48 h: 5
Karakousis PC et al. <i>J. Exp. Med.</i> 2004 200: 647-657	Hollow fiber subcutaneous implant in mice	10 d: 3

TABLE 1-continued

Sources, experimental models, and scoring criteria for <i>M. tuberculosis</i> DNA microarray gene expression and genome-wide gene knock-out (growth phase essentiality).		
Reference	Experimental model	Timepoint: Maximum score ^a
Talaat AM et al. <i>Proc. Natl. Acad. Sci. U.S.A</i> 2004, 101: 4602-4607	Infection of mice. MTB harvested from lung ^b	28 d: 3
Sassetti CM et al. <i>Mol. Microbiol.</i> 2003 48: 77-84	TraSH mutated libraries grown on solid media	14 d: 5
Rengarajan J et al. <i>Proc. Natl. Acad. Sci. U.S.A</i> 2005, 102: 8327-8332	Infection of mouse macrophages, +/- γ -INF with TraSH mutated libraries of <i>M. tuberculosis</i>	7 d: 5
Sassetti CM et al. <i>Proc. Natl. Acad. Sci. U.S.A</i> 2003 100: 12989-12994	C57BL/6J mice infected with TraSH mutated libraries of <i>M. tuberculosis</i>	7, 14, 28 and 56 d: 5

^aMaximum score based on relevance as a dormancy model; h = hour; d = day.

^bRatio of *M. tuberculosis* from Balb/c lung to MTB in aerated culture for 28 d.

#Wayne LG and Hayes LG *Infect. Immun.* 1996 64: 2062-2069

[0591] Step 2—In applying the second criterion, the rank order of gene expression, gene scores from each dataset were ordered from highest to lowest based on expression ratio (fold expression in the experimental condition versus cells in log-phase liquid culture). The highest scoring gene received the maximum score for that particular dataset (listed in column 3 of Table 1. (e.g. 5, 4 . . . , 1 point)). The score was decreased by 0.005 points for each gene in order until zero, or the end of the data set was reached. Thus when the maximum score was 4 points, the 100th ranked gene would receive a score of 3.500. For a maximum score of 5 points, 1000 genes or 25% of the *M. tuberculosis* genome received a score. For experiments where data from multiple time points were collected, the maximum score across all time points was used as the final score.

[0592] In Step 3 scores for each gene in each of the experimental conditions were collected into a Microsoft Access database. Reference fields were added to facilitate prioritisation, such as the Refseq ID, Genbank function, Genbank note, Tuberculist classification, and KEGG and Sanger Center links. By combining the data from different studies and sources, a consensus view was reached about the particular genes and pathways most critical for survival in the dormant state. In Step 4, a prioritised list of therapeutic targets was derived utilising the top 400 scoring genes (~10% of the genome) supplemented by expert computational and manual analysis of biochemical pathways, enzymology, drug tractability, homology to human genes and other prior knowledge. The great majority of the high scoring genes come from the subset where two or three of the groups intersect.

[0593] In Step 5, the identification of subcellular localisation of *M. tuberculosis* proteins encoded by genes, was carried out on the entire genome. The heuristic used for membrane protein prediction is described in Chalker et al. *J. Bacteriol.* 2001 183:1259-1268. Average hydropathy profiles (H) (von Heijne G *J. Mol. Biol.* 1992 225:487-494) were generated using GES hydropathy values (Engelman D M et al. *Annu. Rev. Biophys. Biophys. Chem.* 1986 15:321-353) weighted using a trapezoid window. Using a process similar to the initial steps of the TopPred II algorithm (Claros M G et al. *Comput. Appl. Biosci.* 1994 10:685-686), helical transmembrane segments (TMS) were predicted for each peptide sequence by selecting 19 amino acids centered on the highest

H value (MaxH), masking these from further consideration, and repeating the process until no peaks with a H of >0.5 remained. Subcellular locations were assigned based on the peak MaxH value, number of segments with a H of >1.0, and distribution and peak H values of the putative TMS. A MaxH cutoff of 1.15 was chosen to maximize the discrimination between two SwissProtein release 34 test datasets containing transmembrane and cytoplasmic proteins, respectively (Boyd D et al. *Protein Sci.* 1998 7:201-205). Proteins with a MaxH of <1.15 were classified as cytoplasmic, while those with a MaxH of >1.15 and at least three possible TMS were classified as membrane proteins. Anchored proteins were defined as having exactly two TMS, one starting before amino acid (aa) 35 and one having a H of >1.15 with the other having a H not lower than 0.5. SignalP with Gram positive settings was specifically used for *M. bacterium* to identify secreted proteins amongst those classified as either cytoplasmic or “unknown” in the heuristic analysis (Nielsen H et al. *Protein Eng.* 1997 10:1-6).

[0594] Rv2386c ranked very high as a vaccine antigen according to several criteria:

[0595] (i) Rv2386c is consistently up-regulated across all models of dormancy. Among the entire suite of 3999 genes scored in the meta-analysis, Rv2386c was ranked 120th as one of the top 10% of over-expressed genes across all dormancy models. The up-regulated score for Rv2386c was 13.165 which favourably compared with the top gene score of 22.28. Rv2386c was minimally down-regulated, scoring only slightly less than 0 (-2.316 compared to -18.13 for the most widely down regulated gene).

[0596] (ii) The scavenging of iron from macrophages is crucial for *M. tuberculosis* survival and infectivity. Small molecules called siderophores function in bacteria to bind iron for intra-cellular uptake, MbtI catalyses the first step in the formation of the only *M. tuberculosis* siderophore, mycobactin T, the biosynthesis of which depends on the production of salicylate from chorismate (Harrison et al. *J. Bacteria* 2006 188:6081-6091). Expression of MbtI is induced during *M. tuberculosis* infection of human THP-1 macrophages (Gold et al. *Mol. Microbiol.* 2001 42:851-865). The 3D protein structure is available and the mechanism of enzymatic

action MbtI has been well-studied (Zwahlen et al. *Biochemistry* 2007 46:954-964). Rv2386c ranked high in essentiality scoring and is required by *M. tuberculosis* for survival in in vitro growth models (scoring 2.6 out of a possible scoring of 5).

[0597] (iii) Subcellular localisation predicted that the Rv2386c protein is secreted and thus has significant extracellular exposure, indicating suitability as a vaccine target.

Example 2

Rv2386c Epitope Identification

Method

[0598] T cell epitope prediction was based on the following approaches:

Results

[0599]

TABLE 2

Putative Rv2386c human CD4 + T cell epitopes				
Putative CD4 epitope number	Amino acid position	Epitope sequence	SEQ ID No:	HLA allele
1	54	WVLAAGVQA	SEQ ID No: 30	DRB1_0401
2	55	VLAAGVQAM	SEQ ID No: 31	DRB1_0301, DRB1_1301

Prediction	Name	URL/References
CD4 and CD8	Multipred	website: antigen.i2r.a-star.edu.sg/multipred/ Zhang, G. L., Khan, A. M., Srinivasan, K. N., August, J. T. and Brusic, V. (2005) "MULTIPRED: a computational system for prediction of promiscuous HLA binding peptides" <i>Nucleic Acids Res.</i> 33, W172-W179.
	SVMHC	website: www.bs.informatik.uni-tuebingen.de/SVMHC "Prediction of MHC class I binding peptides, using SVMHC." Pierre Dönnes and Arne Elofsson in: <i>BMC Bioinformatics</i> 2002 3: 25
CD4	ProPred	website: www.imtech.res.in/raghava/propred/ Singh, H. and Raghava, G. P. S. (2001) "ProPred: Prediction of HLA-DR binding sites." <i>Bioinformatics</i> , 17(12), 1236-37.
	Tepitope2	In house program based on: H. Bian, J. Hammer (2004) "Discovery of promiscuous HLA-II-restricted T cell epitopes with TEPITOPE." <i>Methods</i> 34: 468-75
CD8	nHLA	website: www.imtech.res.in/raghava/nhlapred/ Bhasin M. and Raghava G P S (2006) "A hybrid approach for predicting promiscuous MHC class I restricted T cell epitopes"; <i>J. Biosci.</i> 32: 31-42
	NetCTL	website: www.cbs.dtu.dk/services/NetCTL/ "An integrative approach to CTL epitope prediction. A combined algorithm integrating MHC-I binding, TAP transport efficiency, and proteasomal cleavage predictions." Larsen M. V., Lundegaard C., Kasper Lamberth, Buus S., Brunak S., Lund O., and Nielsen M. <i>European Journal of Immunology</i> . 35(8): 2295-303. 2005
	EpiJen	website: www.jenner.ac.uk/EpiJen/ Doytchinova, I. A., P. Guan, D. R. Flower. "EpiJen: a server for multi-step T cell epitope prediction." <i>BMC Bioinformatics</i> , 2006, 7, 131.
	SyFpeithi	website: www.syfpeithi.de/Scripts/MHCServer.dll/EpitopePrediction.htm Hans-Georg Rammensee, Jutta Bachmann, Niels Nikolaus Emmerich, Oskar Alexander Bachor, Stefan Stevanovic: "SYFPEITHI: database for MHC ligands and peptide motifs." <i>Immunogenetics</i> (1999) 50: 213-219
	PredTAP	website: antigen.i2r.a-star.edu.sg/predTAP/ Zhang, G. L., Petrovsky, N., Kwok, C. K., August, J. T. and Brusic, V. (2006) "PRED ^{TAP} : a system for prediction of peptide binding to the human transporter associated with antigen processing." <i>Immunome Res.</i> 2(1), 3.
	PAPROC	website: www.paproc2.de/paproc1/paproc1.html C. Kuttler, A. K. Nussbaum, T. P. Dick, H.-G. Rammensee, H. Schild, K. P. Hader, "An algorithm for the prediction of proteasomal cleavages", <i>J. Mol. Biol.</i> 298 (2000), 417-429 A. K. Nussbaum, C. Kuttler, K. P. Hader, H.-G. Rammensee, H. Schild, "PAPROC: A Prediction Algorithm for Proteasomal Cleavages available on the WWW", <i>Immunogenetics</i> 53 (2001), 87-94

TABLE 2-continued

Putative Rv2386c human CD4 + T cell epitopes				
Putative CD4 epitope number	Amino acid position	Epitope sequence	SEQ ID No:	HLA allele
3	73	VIRDGVTRR SEQ	ID No: 32	DRB1_0301, DRB1_0401
4	125	LAPHTPLAR SEQ	ID No: 33	DRB1_1301
5	148	IRLFDAGIR SEQ	ID No: 34	DRB1_1101, DRB1_1301, DRB1_1501
6	155	IRHREAIR SEQ	ID No: 35	DRB1_0801, DRB1_1301
7	164	LLATGVREV SEQ	ID No: 36	DRB1_1301
8	187	FRRRVAVAV SEQ	ID No: 37	DRB1_0101, DRB1_0801
9	216	FAIDFPLTY SEQ	ID No: 38	DRB1_0301, DRB1_0401
10	220	FPLTYRLGR SEQ	ID No: 39	DRB1_1101
11	224	YRLGRRHNT SEQ	ID No: 40	DRB1_0801, DRB1_1101, DRB1_1501
12	234	VRSPLLQLG SEQ	ID No: 41	DRB1_1301
13	238	LLQLGGIRA SEQ	ID No: 42	DRB1_1501

TABLE 2-continued

Putative Rv2386c human CD4 + T cell epitopes				
Putative CD4 epitope number	Amino acid position	Epitope sequence	SEQ ID No:	HLA allele
14	253	LVTAVRADG SEQ	ID No: 43	DRB1_1301
15	257	VRADGVVIT SEQ	ID No: 44	DRB1_0301, DRB1_0401
16	262	VVITEPLAG SEQ	ID No: 45	DRB1_0301, DRB1_0401, DRB1_1301, DRB1_1501
17	295	IVEHAISVR SEQ	ID No: 46	DRB1_1301
18	321	IDFMTVRER SEQ	ID No: 47	DRB1_1301
19	375	FRLDECPRG SEQ	ID No: 48	DRB1_0301, DRB1_0401
20	384	LYSGAVVML SEQ	ID No: 49	DRB1_0301
21	385	YSGAVVMLS SEQ	ID No: 50	DRB1_0401, DRB1_1101
22	389	VVMLSADGG SEQ	ID No: 51	DRB1_0401, DRB1_1301
23	415	WLRAGAGII SEQ	ID No: 52	DRB1_0101

TABLE 3

Putative Rv2386c human CD8 + T cell epitopes				
Putative CD8 epitope number	Amino acid position	Epitope sequence	SEQ ID No:	HLA allele
1	3	ELSVATGAV SEQ	ID No: 53	A_0201
2	5	SVATGAVST SEQ	ID No: 54	A_0201
3	18	IPMPAGVNP SEQ	ID No: 55	B_0702, B51, Cw_0401
4	19	PMPAGVNPA SEQ	ID No: 56	A_0201
5	21	PAGVNPADL SEQ	ID No: 57	A_2402, B_3501
6	23	GVNPADLAA SEQ	ID No: 58	A3
7	25	NPADLAAEL SEQ	ID No: 59	A24, B_0702, B44, Cw_0401, Cw_0602, B7, B_3501, B51
8	28	DLAAELAAV SEQ	ID No: 60	A_0201
9	33	LAADVTEVS SEQ	ID No: 61	A2, A_0201, B51
10	37	VTEVDVDEY SEQ	ID No: 62	A1, A_0101, A_0301
11	38	TESVDVDEYL SEQ	ID No: 63	B44
12	40	SVDEYLLY SEQ	ID No: 64	A1, A_0101, A3
13	48	YECGGQWVL SEQ	ID No: 65	A24, B_4403, B51, Cw_0401, B44

TABLE 3-continued

Putative Rv2386c human CD8 + T cell epitopes					
Putative CD8 epitope number	Amino acid position	Epitope sequence	SEQ ID No:	HLA allele	
14	52	GQWVLAAGV	SEQ ID No: 66	A2, A_0201	
15	55	VLAAGVQAM	SEQ ID No: 67	A2, B8	
16	65	ELDSDELRV	SEQ ID No: 68	A_0201	
17	67	DSDELRVIR	SEQ ID No: 69	A_0101, A_0301	
18	82	QQWSGRPGA	SEQ ID No: 70	A_0201, A_0301	
19	86	GRPGAALGE	SEQ ID No: 71	A3, B8	
20	87	RPGAALGEA	SEQ ID No: 72	B7, B_0702, B_3501, B51	
21	91	ALGEAVDRL	SEQ ID No: 73	A2, A_0201	
22	93	GEAVDRLLL	SEQ ID No: 74	B44	
23	106	AFGWVAFEF	SEQ ID No: 75	A24	
24	124	RLAPHTPLA	SEQ ID No: 76	A2, A_0201, A3	
25	125	LAPHTPLAR	SEQ ID No: 77	A_0101, A_0301	
26	126	APHTPLARV	SEQ ID No: 78	B7, B_3501, B51	
27	127	PHTPLARVF	SEQ ID No: 79	A24, B44	
28	130	PLARVFSPR	SEQ ID No: 80	A_0101, A3, A_0301	
29	133	RVFSPRTRI	SEQ ID No: 81	B7	
30	134	VFSPRTRIM	SEQ ID No: 82	A24, B8	
31	143	VSEKEIRLF	SEQ ID No: 83	A1, A24	
32	153	AGIRHREAI	SEQ ID No: 84	B8, B51	
33	164	LLATGVREV	SEQ ID No: 85	A2, A_0201	
34	170	REVPQRSRV	SEQ ID No: 86	B44	
35	172	VPQRSVDV	SEQ ID No: 87	B7, B8	
36	180	VSDDPGFR	SEQ ID No: 88	A_0101, A_0301	
37	183	DPSGFRRRV	SEQ ID No: 89	B_3501, B51	
38	185	SGFRRRVAV	SEQ ID No: 90	B8, B51	
39	186	GFRRRVAVA	SEQ ID No: 91	A_0301, B8	
40	187	FRRRVAVAV	SEQ ID No: 92	A3, B8, B51	
41	190	RVAVAVDEI	SEQ ID No: 93	A24, B7	
42	198	IAAGRYHKV	SEQ ID No: 94	B8, B51	
43	200	AGRYHKVIL	SEQ ID No: 95	B7, B8	
44	202	RYHKVILSR	SEQ ID No: 96	A3, A24	
45	206	VILSRCVEV	SEQ ID No: 97	A2, A_0201	
46	214	VPFAIDFPL	SEQ ID No: 98	B7, B_3501, B51	
47	216	FAIDFPLTY	SEQ ID No: 99	A1, A_0101, B_3501, B51	
48	218	IDFPLTYRL	SEQ ID No: 100	B44	

TABLE 3-continued

Putative Rv2386c human CD8 + T cell epitopes					
Putative CD8 epitope number	Amino acid position	Epitope sequence	SEQ ID No:	HLA allele	
49	226	LGRRHNTPV	SEQ ID No: 101	B8, B51	
50	228	RRHNTPVRS	SEQ ID No: 102	A3, B8	
51	231	NTPVRSFLL	SEQ ID No: 103	A_0101, A24	
52	233	PVRSFLLQL	SEQ ID No: 104	A_0201, B7	
53	245	RALGYSPLE	SEQ ID No: 105	A2, A_0201, B_3501, B51	
54	246	ALGYSPELV	SEQ ID No: 106	A_0101, A2, A_0201	
55	249	YSPELVTAV	SEQ ID No: 107	A2	
56	250	SPELVTAVR	SEQ ID No: 108	B_0702, B_3501, B51	
57	256	AVRADGVVI	SEQ ID No: 109	A3, A_0301, B7	
58	264	ITEPLAGTR	SEQ ID No: 110	A_0101, A_0301	
59	266	EPLAGTRAL	SEQ ID No: 111	B7, B8, B_3501, B51	
60	270	GTRALGRGP	SEQ ID No: 112	A3, B8	
61	272	RALGRGPAI	SEQ ID No: 113	A24, B7, B51	
62	288	LESNSKEIV	SEQ ID No: 114	B44	
63	294	EIVEHAISV	SEQ ID No: 115	A_0201	
64	295	IVEHAISVR	SEQ ID No: 116	A_0101	
65	298	HAISVRSSL	SEQ ID No: 117	B7, B8, B_3501	
66	301	SVRSSLEEI	SEQ ID No: 118	B7	
67	305	SLEEITDIA	SEQ ID No: 119	A2	
68	311	DIAEPGSAA	SEQ ID No: 120	A_0101, A_0301	
69	313	AEPGSAAVI	SEQ ID No: 121	B44	
70	327	REGSVQHL	SEQ ID No: 122	B7, B44	
71	331	SVQHLGSTI	SEQ ID No: 123	A24, B7	
72	342	RLDPSSDRM	SEQ ID No: 124	A2, A_0201	
73	344	DPSSDRMAA	SEQ ID No: 125	B7, B_3501	
74	346	SSDRMAALE	SEQ ID No: 126	A1, B8	
75	348	DRMAALEAL	SEQ ID No: 127	B7, Cw_0401, Cw_0602	
76	349	RMAALEALF	SEQ ID No: 128	A24	
77	351	AALEALFPA	SEQ ID No: 129	A2, A_0201, B_3501	
78	352	ALEALFPAV	SEQ ID No: 130	A_0101, A2, A_0201	
79	353	LEALFPAVT	SEQ ID No: 131	B8, B_4403	
80	357	FPAVTASGI	SEQ ID No: 132	B7, B8, B_3501, B51	
81	359	AVTASGIPK	SEQ ID No: 133	A3, A_0301, B7	
82	360	VTASGIPKA	SEQ ID No: 134	A_0201	

TABLE 3-continued

Putative Rv2386c human CD8 + T cell epitopes				
Putative CD8 epitope number	Amino acid position	Epitope sequence	SEQ ID No:	HLA allele
83	364	GIPKAAGVE	SEQ ID No: 135	A_0301, B8
84	365	IPKAAGVEA	SEQ ID No: 136	B7, B_3501
85	367	KAAGVEAIF	SEQ ID No: 137	A24, B_3501
87	369	AGVEAIFRL	SEQ ID No: 138	A_0201, B51
87	376	RLDECPRGL	SEQ ID No: 139	A2, A_0201
88	380	CPRGLYSGA	SEQ ID No: 140	B7, B_3501
89	383	GLYSGAVVM	SEQ ID No: 141	A2, A_0201, A_0301, A3
90	384	LYSGAVVML	SEQ ID No: 142	A24, B44
91	390	VMLSADGGL	SEQ ID No: 143	A2, A_0201, A24
92	397	GLDAALTLR	SEQ ID No: 144	A_0101, A3, A_0301
93	398	LDAALTLRA	SEQ ID No: 145	A_0201, B_4403
94	400	AALTLRAAY	SEQ ID No: 146	A_0101, A_0301, B_3501
95	402	LTLRAAYQV	SEQ ID No: 147	A_0201
96	407	AYQVGGRTW	SEQ ID No: 148	A24
97	414	TWLRAGAGI	SEQ ID No: 149	A24
98	424	EESEPEREF	SEQ ID No: 150	B44
99	430	REFEETCEK	SEQ ID No: 151	B44
100	438	KLSTLTPYL	SEQ ID No: 152	A2, A_0201
101	440	STLTPYLVA	SEQ ID No: 153	A_0101, A_0201
102	441	TLTPYLVAR	SEQ ID No: 154	A3, A_0301

[0600] As can be seen from Tables 2 and 3, Rv2386c contains a number of predicted CD4+ and CD8 T cell epitopes. Furthermore, this information suggests that the protein carries epitopes that can be recognised by HLAs which occur worldwide (that is HLAs from Caucasian, African, Asian or Latin-American individuals—see website at www.allele-frequencies.net).

Example 3

H37Rv Homologues

[0601] Rv2386c sequences from a number of *M. tuberculosis* strains and BCG were identified using BLASTP searches of GenBank (H37Rv reference sequence accession number YP_177877.1).

Strain	Accession Number	% identity
CDC1551	NP_336935.1	100
F11	YP_001288342.1	100
Haarlem	ZP_02247771.1	100

-continued

Strain	Accession Number	% identity
C	ZP_00878160.1	99
BCG	CAL72388.1	100

[0602] Alignment of the homologue sequences indicates a high level of identity.

Biological Assays

Quantification of T Cell Responses to Rv2386c

[0603] Polypeptides may be screened for their ability to activate T-cells (induction of proliferation and/or production of cytokines) in peripheral blood mononuclear cell (PBMC) or in whole blood preparations from infected (such as latently infected) individuals.

[0604] Latently infected individuals are usually identified by a skin test that has a diameter above 10 mm and without symptoms, with no Mtb positive culture, with a negative sputum negative and with no lesion (as detected by chest X-Ray).

[0605] A range of in vitro assays can be used based on PBMC samples or whole blood: after restimulation in presence of the antigen (or variant/immunogenic fragment thereof as appropriate) the proliferation of the cells may be determined (as measured by CFSE/flow cytometry) or the production of cytokines quantified (present in the supernatant of cultured cells and measured by ELISA, or, after intracellular staining of CD4 and CD8 T cells and analysis by flow cytometry).

[0606] For example, PBMC samples may be obtained from heparinised whole blood by Ficoll-Hypaque density gradient centrifugation following standard procedures. The cells may then be washed and cryopreserved in liquid nitrogen until testing (for further details see Lalvani A et al. *J. Infect. Dis.* 1999 180:1656-1664).

T Cell Proliferation

[0607] The specific immune response may be characterised by performing lymphocyte proliferation analysis using the tritiated thymidine. This technique assesses the cellular expansion upon in vitro stimulation to an antigen. In practice, cell proliferation is determined by estimating incorporation of tritiated-thymidine into DNA, a process closely related to underlying changes in cell number.

[0608] More suitably, lymphocyte proliferation may be performed using the succinimidyl ester of carboxyfluorescein diacetate (CFSE). CFSE spontaneously and irreversibly couples to both intracellular and cell surface proteins by reaction with lysine side chains and other available amine groups. When lymphocyte cells divide, CFSE labelling is distributed equally between the daughter cells, which are therefore half as fluorescent as the parents. As a result, halving of cellular fluorescence intensity marks each successive generation in a population of proliferating cells and is readily followed by flow cytometry (for further details see Hodgkins, P D et al *J. Exp. Med.* 1996 184:277-281).

[0609] Practically, after thawing, PMBC may be washed and stained with CFSE before being cultivated (2×10^5 cells) for 72 hrs with 10 ug/ml of antigen in culture media (RPMI-1640 supplemented with glutamine, non essential amino acid, pyruvate and heat inactivated human AB serum). Cells may then then harvested and their phenotype characterised using surface staining to identify memory CD8 and CD4+ T-Cells. Subsequently, flow cytometry analysis can be used to indicate the extent of lymphocyte proliferation in response to each antigen (proportion of cells with decreased CFSE intensity upon in vitro stimulation).

Cytokine Production

[0610] IFN- γ production (or the production of other cytokines such as e.g. IL2, TNF-alpha, IL5, IL12 etc) may be measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates may be coated with a mouse monoclonal antibody directed to human IFN- γ (PharMingen, San Diego, Calif.) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are then washed, for example, six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN- γ serum, for example, diluted 1:3000 in PBS/10% normal goat serum may be added to each well. The plates are then

incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, Mo.) may be added, for example, at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction may be stopped after 20 min with 1 N sulfuric acid. Optical density can then be determined at 450 nm using 570 nm as a reference wavelength. Typically, fractions that result in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone may be considered positive.

Example 4

Immunogenicity in CB6F1 Mice

[0611] The immunogenicity of the antigen was evaluated in CB6F1 mice (first generation cross of BALB/c and C57BL/6 mice).

[0612] CB6F1 mice were immunised intramuscularly three times (on day 0, day 14 and day 28) with 0.5 ug or 2 ug of protein antigen in combination with the Adjuvant System AS01E (a liposomal adjuvant formulation comprising 3D-MPL and QS21).

[0613] The experimental design was as follows:

Group	Day 0	Day 14	Day 28
1	2 ug Rv2386c/AS01E	2 ug Rv2386c/AS01E	2 ug Rv2386c/AS01E
2	0.5 ug Rv2386c/AS01E	0.5 ug Rv2386c/AS01E	0.5 ug Rv2386c/AS01E

[0614] A total of 24 mice were used in each protocol group.

[0615] Peripheral blood lymphocytes (PBL) were collected and pooled on day 21 (i.e. 7 days post second immunisation) and day 35 (i.e. 7 days post third immunisation) and the antigen-specific CD4 & CD8 T cell responses (as determined by CD4 or CD8 T cells producing IL-2 and/or IFN-gamma and/or TNF-alpha) were measured by flow cytometry after overnight in vitro restimulation with pools of 15mer peptides covering the sequences of interest. The detection of mouse T cells that express IL-2 and/or IFN-gamma and/or TNF-alpha was done by using short-term antigen-driven in vitro amplification of cytokine expression.

[0616] Briefly, PharmLyse solution (BD-Pharmingen) was added to heparinised mouse peripheral blood in order to lyse the red blood cells. The PBLs (Peripheral Blood Lymphocytes) obtained were washed and then incubated in the presence of a pool of 15-mer peptides—overlapping by 11 amino acids—covering the sequence of the antigen of interest and of 1 ug/ml of antibodies to CD28 and CD49d (BD-Pharmingen). Each 15-mer peptide was used at a final concentration of 1 ug/ml. Medium controls were also stimulated with antibodies to CD28 and CD49d.

[0617] The cytokine secretion blocking compound brefeldin-A (BD-Pharmingen) was added 2 h after the onset of the cultures at 37° C., 5% CO₂ and the cells maintained at 37° C., 5% CO₂ for 4 additional hours followed by overnight incubation at +4° C.

[0618] Cells were then harvested and stained with Pacific Blue-coupled anti-CD4 (BD—clone RM4-5, BD-Pharmingen).

gen) and peridinin chlorophyll A protein (PerCp) cyanin5.5 (Cy5.5)-coupled anti-CD8 alpha (clone 53-6.7, BD-Pharmingen) antibodies.

[0619] Cells were then washed, fixed, permeabilised (Cytofix-cytoperm kit, BD-Pharmingen) and stained with allophycocyanin-coupled anti-IFN-g antibodies (clone XMG1.2, BDPharmingen), fluorescein isothiocyanate (FITC)-coupled anti-IL-2 antibodies (clone JES 6-5H4, Beckman Coulter) and phycoerythrin (PE)-coupled anti-TNF alpha antibodies (clone MP6-XT22, BDPharmingen). After final washes, stained cells were analysed on a LSR II flow cytometer (Beckton-Dickinson). A minimum number of 10,000 cells were acquired in the CD8+ subset.

[0620] For further background see Walzer T et al *Cell Immunol.* 2000 206(1):16-25 and Maecker H T et al *J. Immunol. Methods* 2001 255(1-2):27-40.

[0621] As negative controls, some cells were also cultured overnight in vitro in culture medium (unstimulated). The antigen-specific responses were calculated by subtracting the average cytokine response produced by unstimulated cells from the average cytokine response produced by the peptide-stimulated cells.

[0622] At each timepoint and for each group, the data was collected from 4 pools of 6 mice each. The data below is presented as the % of CD4 or CD8 T cells producing IL-2 and/or IFN-gamma and/or TNF-alpha. Each individual pool of mice is plotted (triangles) as well as the average value of the group (bar).

[0623] FIG. 1 shows that on day 21 (i.e. 7 days post second immunisation), Rv2386c-specific CD4 and CD8 T cell responses are detected in mice immunised with either dose of Rv2386c/AS01E. The levels of Rv2386c-specific CD4 T cell responses are similar regardless of the immunising dose of Rv2386. In contrast, mice immunised with 2 ug Rv2386c/AS01E displayed higher Rv2386c-specific CD8 T cell responses than mice immunised with 0.5 ug Rv2386c/AS01E.

[0624] FIG. 2 shows the cytokine profile of CD4 T cell response from the Rv2386c peptide pool-stimulated PBL (not medium removed) on day 21 (i.e. 7 days post second immunisation).

[0625] FIG. 3 shows the cytokine profile of CD8 T cell response from the Rv2386c peptide pool-stimulated PBL (not medium removed) on day 21 (i.e. 7 days post second immunisation).

[0626] FIG. 4 shows that on day 35 (i.e. 7 days post third immunisation), Rv2386c-specific CD4 and CD8 T cell responses are detected in mice immunised with either dose of Rv2386c/AS01E. The levels of Rv2386c-specific CD4 T cell responses are similar regardless of the immunising dose of Rv2386. In contrast, mice immunised with 2 ug Rv2386c/AS01E displayed higher Rv2386c-specific CD8 T cell responses than mice immunised with 0.5 ug Rv2386c/AS01E. The third immunisation dose increases the CD4 T cell response but not the CD8 T cell response.

[0627] FIG. 5 shows the cytokine profile of CD4 T cell response from the Rv2386c peptide pool-stimulated PBL (not medium removed) on day 35 (i.e. 7 days post third immunisation).

[0628] FIG. 6 shows the cytokine profile of CD8 T cell response from the Rv2386c peptide pool-stimulated PBL (not medium removed) on day 35 (i.e. 7 days post third immunisation).

Example 5

Immunogenicity in C57BL/6 Mice

[0629] The immunogenicity of the antigen was also evaluated in C57BL/6 mice. C57BL/6 mice were immunised intramuscularly three times (on day 0, day 14 and day 28) with 1 ug or 4 ug of protein antigen in combination with a the Adjuvant System AS01E (a liposomal adjuvant formulation comprising 3D-MPL and QS21).

[0630] The experimental design was the following:

Group	Day 0	Day 14	Day 28
1	4 ug Rv2386c/AS01E	4 ug Rv2386c/AS01E	4 ug Rv2386c/AS01E
2	1 ug Rv2386c/AS01E	1 ug Rv2386c/AS01E	1 ug Rv2386c/AS01E

[0631] Peripheral blood lymphocytes (PBL) were collected and pooled on day 21 (i.e. 7 days post second immunisation) and day 35 (i.e. 7 days post third immunisation) and the antigen-specific CD4 & CD8 T cell responses (as determined by CD4 or CD8 T cells producing IL-2 and/or IFN-gamma and/or TNF-alpha) were measured by flow cytometry after overnight in vitro restimulation with pools of 15mer peptides covering the sequences of interest. The procedure followed was as described previously.

[0632] As negative controls, some cells were also cultured overnight in vitro in culture medium (unstimulated). The antigen-specific responses were calculated by subtracting the average cytokine response produced by unstimulated cells from the average cytokine response produced by the peptide-stimulated cells.

[0633] At each timepoint and for each group, the data was collected from 4 pools of 6 mice each. The data below is presented as the % of CD4 or CD8 T cells producing IL-2 and/or IFN-gamma and/or TNF-alpha. Each individual pool of mice is plotted (triangles) as well as the average value of the group (bar).

[0634] FIG. 7 shows that on day 21 (i.e. 7 days post second immunisation), Rv2386c-specific CD4 and CD8 T cell responses are detected in mice immunised with either dose of Rv2386c/AS01E. The levels of Rv2386c-specific CD4 T cell responses are similar regardless of the immunising dose of Rv2386c. In contrast, mice immunised with 4 ug Rv2386c/AS01E displayed higher Rv2386c-specific CD8 T cell responses than mice immunized with 1 ug Rv2386c/AS01E. Due to technical difficulties, data for the first pool of cells at the 4 ug dose are not available.

[0635] FIG. 8 shows the cytokine profile of CD4 T cell response from the Rv2386c peptide pool-stimulated PBL (not medium removed) on day 21 (i.e. 7 days post second immunisation). Due to technical difficulties, data for the first pool of cells at the 4 ug dose are not available.

[0636] FIG. 9 shows the cytokine profile of CD8 T cell response from the Rv2386c peptide pool-stimulated PBL (not medium removed) on day 21 (i.e. 7 days post second immunisation). Due to technical difficulties, data for the first pool of cells at the 4 ug dose are not available.

[0637] Immunological data for day 35 were not yet available at the time this application was prepared.

[0638] In conclusion it may be noted that the Rv2386c antigen is capable of eliciting an immune response in both

CB6F1 and C57BL/6 mice. Furthermore, the profile of cytokine production indicates that a large proportion of antigen-specific T-cells express a plurality of Th1 associated cytokines (i.e. a polyfunctional T-cell response is elicited). Importantly both CD4 and CD8 antigen-specific T-cells are present after immunisation, CD8 cells may be particularly important in a latent TB scenario. Rv2386c may therefore be expected to be of substantial value in the prevention, treatment and diagnosis of latent tuberculosis infection.

[0639] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifi-

cations may be made thereto without departing from the spirit or scope of the appended claims.

[0640] All references referred to in this application, including patents and patent applications, are incorporated herein by reference to the fullest extent possible as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0641] Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer, step, group of integers or group of steps but not to the exclusion of any other integer, step, group of integers or group of steps.

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Glu Cys Asp Gly Gln Trp Val Leu Ala Ala Gly Val Gln Ala Met Val
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Glu Leu Asp Ser Asp Glu Leu Arg Val Ile Arg Asp Gly Val Thr Arg
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Arg Gln Gln Trp Ser Gly Arg Pro Gly Ala Ala Leu Gly Glu Ala Val
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Asp Arg Leu Leu Leu Glu Thr Asp Gln Ala Phe Gly Trp Val Ala Phe
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Glu Phe Gly Val His Arg Tyr Gly Leu Gln Gln Arg Leu Ala Pro His
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Thr Pro Leu Ala Arg Val Phe Ser Pro Arg Thr Arg Ile Met Val Ser
      130            135            140

Glu Lys Glu Ile Arg Leu Phe Asp Ala Gly Ile Arg His Arg Glu Ala
      145            150            155            160

Ile Asp Arg Leu Leu Ala Thr Gly Val Arg Glu Val Pro Gln Ser Arg
      165            170            175

Ser Val Asp Val Ser Asp Asp Pro Ser Gly Phe Arg Arg Arg Val Ala
      180            185            190

Val Ala Val Asp Glu Ile Ala Ala Gly Arg Tyr His Lys Val Ile Leu
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Ser Arg Cys Val Glu Val Pro Phe Ala Ile Asp Phe Pro Leu Thr Tyr
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 Ala Leu Gly Arg Gly Pro Ala Ile Asp Arg Leu Ala Arg Asp Asp Leu
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 Glu Ser Asn Ser Lys Glu Ile Val Glu His Ala Ile Ser Val Arg Ser
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 Ser Leu Glu Glu Ile Thr Asp Ile Ala Glu Pro Gly Ser Ala Ala Val
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 325 330 335
 Ser Thr Ile Arg Ala Arg Leu Asp Pro Ser Ser Asp Arg Met Ala Ala
 340 345 350
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 Ala Gly Val Glu Ala Ile Phe Arg Leu Asp Glu Cys Pro Arg Gly Leu
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 Tyr Ser Gly Ala Val Val Met Leu Ser Ala Asp Gly Gly Leu Asp Ala
 385 390 395 400
 Ala Leu Thr Leu Arg Ala Ala Tyr Gln Val Gly Gly Arg Thr Trp Leu
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35          40          45

Glu Cys Asp Gly Gln Trp Val Leu Ala Ala Gly Val Gln Ala Met Val
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Glu Leu Asp Ser Asp Glu Leu Arg Val Ile Arg Asp Gly Val Thr Arg
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Thr Pro Leu Ala Arg Val Phe Ser Pro Arg Thr Arg Ile Met Val Ser
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Glu Lys Glu Ile Arg Leu Phe Asp Ala Gly Ile Arg His Arg Glu Ala
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Ile Asp Arg Leu Leu Ala Thr Gly Val Arg Glu Val Pro Gln Ser Arg
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Glu Ser Asn Ser Lys Glu Ile Val Glu His Ala Ile Ser Val Arg Ser
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Ser Leu Glu Glu Ile Thr Asp Ile Ala Glu Pro Gly Ser Ala Ala Val
 305                310                315                320

Ile Asp Phe Met Thr Val Arg Glu Arg Gly Ser Val Gln His Leu Gly
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Tyr Ser Gly Ala Val Val Met Leu Ser Ala Asp Gly Gly Leu Asp Ala
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      115                      120                      125

Thr Pro Leu Ala Arg Val Phe Ser Pro Arg Thr Arg Ile Met Val Ser
      130                      135                      140

Glu Lys Glu Ile Arg Leu Phe Asp Ala Gly Ile Arg His Arg Glu Ala
      145                      150                      155                      160

Ile Asp Arg Leu Leu Ala Thr Gly Val Arg Glu Val Pro Gln Ser Arg
      165                      170                      175

Ser Val Asp Val Ser Asp Asp Pro Ser Gly Phe Arg Arg Val Ala
      180                      185                      190

Val Ala Val Asp Glu Ile Ala Ala Gly Arg Tyr His Lys Val Ile Leu
      195                      200                      205

Ser Arg Cys Val Glu Val Pro Phe Ala Ile Asp Phe Pro Leu Thr Tyr
      210                      215                      220

Arg Leu Gly Arg Arg His Asn Thr Pro Val Arg Ser Phe Leu Leu Gln
      225                      230                      235                      240

Leu Gly Gly Ile Arg Ala Leu Gly Tyr Ser Pro Glu Leu Val Thr Ala
      245                      250                      255

Val Arg Ala Asp Gly Val Val Ile Thr Glu Pro Leu Ala Gly Thr Arg
      260                      265                      270

Ala Leu Gly Arg Gly Pro Ala Ile Asp Arg Leu Ala Arg Asp Asp Leu
      275                      280                      285

Glu Ser Asn Ser Lys Glu Ile Val Glu His Ala Ile Ser Val Arg Ser
      290                      295                      300

Ser Leu Glu Glu Ile Thr Asp Ile Ala Glu Pro Gly Ser Ala Ala Val
      305                      310                      315                      320

Ile Asp Phe Met Thr Val Arg Glu Arg Gly Ser Val Gln His Leu Gly
      325                      330                      335

Ser Thr Ile Arg Ala Arg Leu Asp Pro Ser Ser Asp Arg Met Ala Ala
      340                      345                      350

Leu Glu Ala Leu Phe Pro Ala Val Thr Ala Ser Gly Ile Pro Lys Ala
      355                      360                      365

Ala Gly Val Glu Ala Ile Phe Arg Leu Asp Glu Cys Pro Arg Gly Leu
      370                      375                      380

Tyr Ser Gly Ala Val Val Met Leu Ser Ala Asp Gly Gly Leu Asp Ala
      385                      390                      395                      400

Ala Leu Thr Leu Arg Ala Ala Tyr Gln Val Gly Gly Arg Thr Trp Leu
      405                      410                      415

Arg Ala Gly Ala Gly Ile Ile Glu Glu Ser Glu Pro Glu Arg Glu Phe
      420                      425                      430

Glu Glu Thr Cys Glu Lys Leu Ser Thr Leu Thr Pro Tyr Leu Val Ala
      435                      440                      445

Arg Gln
      450

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<210> SEQ ID NO 5
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis
<220> FEATURE:

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<221> NAME/KEY: MISC_FEATURE

<223> OTHER INFORMATION: Strain Haarlem A

<400> SEQUENCE: 5

```

Met Ser Glu Leu Ser Val Ala Thr Gly Ala Val Ser Thr Ala Ser Ser
1           5           10           15

Ser Ile Pro Met Pro Ala Gly Val Asn Pro Ala Asp Leu Ala Ala Glu
20          25          30

Leu Ala Ala Val Val Thr Glu Ser Val Asp Glu Asp Tyr Leu Leu Tyr
35          40          45

Glu Cys Asp Gly Gln Trp Val Leu Ala Ala Gly Val Gln Ala Met Val
50          55          60

Glu Leu Asp Ser Asp Glu Leu Arg Val Ile Arg Asp Gly Val Thr Arg
65          70          75          80

Arg Gln Gln Trp Ser Gly Arg Pro Gly Ala Ala Leu Gly Glu Ala Val
85          90          95

Asp Arg Leu Leu Leu Glu Thr Asp Gln Ala Phe Gly Trp Val Ala Phe
100         105         110

Glu Phe Gly Val His Arg Tyr Gly Leu Gln Gln Arg Leu Ala Pro His
115         120         125

Thr Pro Leu Ala Arg Val Phe Ser Pro Arg Thr Arg Ile Met Val Ser
130         135         140

Glu Lys Glu Ile Arg Leu Phe Asp Ala Gly Ile Arg His Arg Glu Ala
145         150         155         160

Ile Asp Arg Leu Leu Ala Thr Gly Val Arg Glu Val Pro Gln Ser Arg
165         170         175

Ser Val Asp Val Ser Asp Asp Pro Ser Gly Phe Arg Arg Arg Val Ala
180         185         190

Val Ala Val Asp Glu Ile Ala Ala Gly Arg Tyr His Lys Val Ile Leu
195         200         205

Ser Arg Cys Val Glu Val Pro Phe Ala Ile Asp Phe Pro Leu Thr Tyr
210         215         220

Arg Leu Gly Arg Arg His Asn Thr Pro Val Arg Ser Phe Leu Leu Gln
225         230         235         240

Leu Gly Gly Ile Arg Ala Leu Gly Tyr Ser Pro Glu Leu Val Thr Ala
245         250         255

Val Arg Ala Asp Gly Val Val Ile Thr Glu Pro Leu Ala Gly Thr Arg
260         265         270

Ala Leu Gly Arg Gly Pro Ala Ile Asp Arg Leu Ala Arg Asp Asp Leu
275         280         285

Glu Ser Asn Ser Lys Glu Ile Val Glu His Ala Ile Ser Val Arg Ser
290         295         300

Ser Leu Glu Glu Ile Thr Asp Ile Ala Glu Pro Gly Ser Ala Ala Val
305         310         315         320

Ile Asp Phe Met Thr Val Arg Glu Arg Gly Ser Val Gln His Leu Gly
325         330         335

Ser Thr Ile Arg Ala Arg Leu Asp Pro Ser Ser Asp Arg Met Ala Ala
340         345         350

Leu Glu Ala Leu Phe Pro Ala Val Thr Ala Ser Gly Ile Pro Lys Ala
355         360         365

Ala Gly Val Glu Ala Ile Phe Arg Leu Asp Glu Cys Pro Arg Gly Leu
370         375         380

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Tyr Ser Gly Ala Val Val Met Leu Ser Ala Asp Gly Gly Leu Asp Ala
385                390                395                400

Ala Leu Thr Leu Arg Ala Ala Tyr Gln Val Gly Gly Arg Thr Trp Leu
                405                410                415

Arg Ala Gly Ala Gly Ile Ile Glu Glu Ser Glu Pro Glu Arg Glu Phe
                420                425                430

Glu Glu Thr Cys Glu Lys Leu Ser Thr Leu Thr Pro Tyr Leu Val Ala
435                440                445

Arg Gln
450

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<210> SEQ ID NO 6
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Strain C

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<400> SEQUENCE: 6

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Met Ser Glu Leu Ser Val Ala Thr Gly Ala Val Ser Thr Ala Ser Ser
1                5                10                15

Ser Ile Pro Met Pro Ala Gly Val Asn Pro Ala Asp Leu Ala Ala Glu
                20                25                30

Leu Ala Ala Val Val Thr Glu Ser Val Asp Glu Asp Tyr Leu Leu Tyr
                35                40                45

Glu Cys Asp Gly Gln Trp Val Leu Ala Ala Gly Val Gln Ala Met Val
50                55                60

Glu Leu Asp Ser Asp Glu Leu Arg Val Ile Arg Asp Gly Val Thr Arg
65                70                75                80

Arg Gln Gln Trp Ser Gly Arg Pro Gly Ala Ala Leu Gly Glu Ala Val
                85                90                95

Asp Arg Leu Leu Leu Glu Thr Asp Gln Ala Phe Gly Trp Val Ala Leu
100               105               110

Glu Phe Gly Val His Arg Tyr Gly Leu Gln Gln Arg Leu Ala Pro His
115               120               125

Thr Pro Leu Ala Arg Val Phe Ser Pro Arg Thr Arg Ile Met Val Ser
130               135               140

Glu Lys Glu Ile Arg Leu Phe Asp Ala Gly Ile Arg His Arg Glu Ala
145               150               155               160

Ile Asp Arg Leu Leu Ala Thr Gly Val Arg Glu Val Pro Gln Ser Arg
165               170               175

Ser Val Asp Val Ser Asp Asp Pro Ser Gly Phe Arg Arg Arg Val Ala
180               185               190

Val Ala Val Asp Glu Ile Ala Ala Gly Arg Tyr His Lys Val Ile Leu
195               200               205

Ser Arg Cys Val Glu Val Pro Phe Ala Ile Asp Phe Pro Leu Thr Tyr
210               215               220

Arg Leu Gly Arg Arg His Asn Thr Pro Val Arg Ser Phe Leu Leu Gln
225               230               235               240

Leu Gly Gly Ile Arg Ala Leu Gly Tyr Ser Pro Glu Leu Val Thr Ala
245               250               255

Val Arg Ala Asp Gly Val Val Ile Thr Glu Pro Leu Ala Gly Thr Arg

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260					265					270						
Ala	Leu	Gly	Arg	Gly	Pro	Ala	Ile	Asp	Arg	Leu	Ala	Arg	Asp	Asp	Leu	
275					280					285						
Glu	Ser	Asn	Ser	Lys	Glu	Ile	Val	Glu	His	Ala	Ile	Ser	Val	Arg	Ser	
290					295					300						
Ser	Leu	Glu	Glu	Ile	Thr	Asp	Ile	Ala	Glu	Pro	Gly	Ser	Ala	Ala	Val	
305					310					315					320	
Ile	Asp	Phe	Met	Thr	Val	Arg	Glu	Arg	Gly	Ser	Val	Gln	His	Leu	Gly	
325					330					335						
Ser	Thr	Ile	Arg	Ala	Arg	Leu	Asp	Pro	Ser	Ser	Asp	Arg	Met	Ala	Ala	
340					345					350						
Leu	Glu	Ala	Leu	Phe	Pro	Ala	Val	Thr	Ala	Ser	Gly	Ile	Pro	Lys	Ala	
355					360					365						
Ala	Gly	Val	Glu	Ala	Ile	Phe	Arg	Leu	Asp	Glu	Cys	Pro	Arg	Gly	Leu	
370					375					380						
Tyr	Ser	Gly	Ala	Val	Val	Met	Leu	Ser	Ala	Asp	Gly	Gly	Leu	Asp	Ala	
385					390					395					400	
Ala	Leu	Thr	Leu	Arg	Ala	Ala	Tyr	Gln	Val	Gly	Gly	Arg	Thr	Trp	Leu	
405					410					415						
Arg	Ala	Gly	Ala	Gly	Ile	Ile	Glu	Glu	Ser	Glu	Pro	Glu	Arg	Glu	Phe	
420					425					430						
Glu	Glu	Thr	Cys	Glu	Lys	Leu	Ser	Thr	Leu	Thr	Pro	Tyr	Leu	Val	Ala	
435					440					445						
Arg	Gln															
450																

<210> SEQ ID NO 7
 <211> LENGTH: 450
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium bovis
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <223> OTHER INFORMATION: Strain BCG

<400> SEQUENCE: 7

Met	Ser	Glu	Leu	Ser	Val	Ala	Thr	Gly	Ala	Val	Ser	Thr	Ala	Ser	Ser
1				5					10					15	
Ser	Ile	Pro	Met	Pro	Ala	Gly	Val	Asn	Pro	Ala	Asp	Leu	Ala	Ala	Glu
		20						25					30		
Leu	Ala	Ala	Val	Val	Thr	Glu	Ser	Val	Asp	Glu	Asp	Tyr	Leu	Leu	Tyr
		35					40					45			
Glu	Cys	Asp	Gly	Gln	Trp	Val	Leu	Ala	Ala	Gly	Val	Gln	Ala	Met	Val
	50					55					60				
Glu	Leu	Asp	Ser	Asp	Glu	Leu	Arg	Val	Ile	Arg	Asp	Gly	Val	Thr	Arg
65					70				75					80	
Arg	Gln	Gln	Trp	Ser	Gly	Arg	Pro	Gly	Ala	Ala	Leu	Gly	Glu	Ala	Val
			85					90						95	
Asp	Arg	Leu	Leu	Glu	Thr	Asp	Gln	Ala	Phe	Gly	Trp	Val	Ala	Phe	
		100					105					110			
Glu	Phe	Gly	Val	His	Arg	Tyr	Gly	Leu	Gln	Gln	Arg	Leu	Ala	Pro	His
	115						120				125				
Thr	Pro	Leu	Ala	Arg	Val	Phe	Ser	Pro	Arg	Thr	Arg	Ile	Met	Val	Ser
	130					135					140				

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Glu Lys Glu Ile Arg Leu Phe Asp Ala Gly Ile Arg His Arg Glu Ala
 145 150 155 160
 Ile Asp Arg Leu Leu Ala Thr Gly Val Arg Glu Val Pro Gln Ser Arg
 165 170 175
 Ser Val Asp Val Ser Asp Asp Pro Ser Gly Phe Arg Arg Arg Val Ala
 180 185 190
 Val Ala Val Asp Glu Ile Ala Ala Gly Arg Tyr His Lys Val Ile Leu
 195 200 205
 Ser Arg Cys Val Glu Val Pro Phe Ala Ile Asp Phe Pro Leu Thr Tyr
 210 215 220
 Arg Leu Gly Arg Arg His Asn Thr Pro Val Arg Ser Phe Leu Leu Gln
 225 230 235 240
 Leu Gly Gly Ile Arg Ala Leu Gly Tyr Ser Pro Glu Leu Val Thr Ala
 245 250 255
 Val Arg Ala Asp Gly Val Val Ile Thr Glu Pro Leu Ala Gly Thr Arg
 260 265 270
 Ala Leu Gly Arg Gly Pro Ala Ile Asp Arg Leu Ala Arg Asp Asp Leu
 275 280 285
 Glu Ser Asn Ser Lys Glu Ile Val Glu His Ala Ile Ser Val Arg Ser
 290 295 300
 Ser Leu Glu Glu Ile Thr Asp Ile Ala Glu Pro Gly Ser Ala Ala Val
 305 310 315 320
 Ile Asp Phe Met Thr Val Arg Glu Arg Gly Ser Val Gln His Leu Gly
 325 330 335
 Ser Thr Ile Arg Ala Arg Leu Asp Pro Ser Ser Asp Arg Met Ala Ala
 340 345 350
 Leu Glu Ala Leu Phe Pro Ala Val Thr Ala Ser Gly Ile Pro Lys Ala
 355 360 365
 Ala Gly Val Glu Ala Ile Phe Arg Leu Asp Glu Cys Pro Arg Gly Leu
 370 375 380
 Tyr Ser Gly Ala Val Val Met Leu Ser Ala Asp Gly Gly Leu Asp Ala
 385 390 395 400
 Ala Leu Thr Leu Arg Ala Ala Tyr Gln Val Gly Gly Arg Thr Trp Leu
 405 410 415
 Arg Ala Gly Ala Gly Ile Ile Glu Glu Ser Glu Pro Glu Arg Glu Phe
 420 425 430
 Glu Glu Thr Cys Glu Lys Leu Ser Thr Leu Thr Pro Tyr Leu Val Ala
 435 440 445
 Arg Gln
 450

<210> SEQ ID NO 8
 <211> LENGTH: 110
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis
 <220> FEATURE:
 <221> NAME/KEY: mat_peptide
 <222> LOCATION: (29)..(110)

<400> SEQUENCE: 8

Met Arg Leu Ser Leu Thr Ala Leu Ser Ala Gly Val Gly Ala Val Ala
 -25 -20 -15 -10
 Met Ser Leu Thr Val Gly Ala Gly Val Ala Ser Ala Asp Pro Val Asp
 -5 -1 1 5

-continued

Ala Val Ile Asn Thr Thr Cys Asn Tyr Gly Gln Val Val Ala Ala Leu
 10 15 20
 Asn Ala Thr Asp Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser Pro Val
 25 30 35
 Ala Gln Ser Tyr Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro Gln Arg
 40 45 50
 Ala Ala Met Ala Ala Gln Leu Gln Ala Val Pro Gly Ala Ala Gln Tyr
 55 60 65
 Ile Gly Leu Val Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr
 70 75 80

<210> SEQ ID NO 9
 <211> LENGTH: 97
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 9

Met Ser Leu Leu Asp Ala His Ile Pro Gln Leu Val Ala Ser Gln Ser
 1 5 10 15
 Ala Phe Ala Ala Lys Ala Gly Leu Met Arg His Thr Ile Gly Gln Ala
 20 25 30
 Glu Gln Ala Ala Met Ser Ala Gln Ala Phe His Gln Gly Glu Ser Ser
 35 40 45
 Ala Ala Phe Gln Ala Ala His Ala Arg Phe Val Ala Ala Ala Ala Lys
 50 55 60
 Val Asn Thr Leu Leu Asp Val Ala Gln Ala Asn Leu Gly Glu Ala Ala
 65 70 75 80
 Gly Thr Tyr Val Ala Ala Asp Ala Ala Ala Ser Thr Tyr Thr Gly
 85 90 95

Phe

<210> SEQ ID NO 10
 <211> LENGTH: 94
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 10

Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met
 1 5 10 15
 Ile Arg Ala Gln Ala Ala Ser Leu Glu Ala Glu His Gln Ala Ile Val
 20 25 30
 Arg Asp Val Leu Ala Ala Gly Asp Phe Trp Gly Gly Ala Gly Ser Val
 35 40 45
 Ala Cys Gln Glu Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile
 50 55 60
 Tyr Glu Gln Ala Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn
 65 70 75 80
 Asn Met Ala Gln Thr Asp Ser Ala Val Gly Ser Ser Trp Ala
 85 90

<210> SEQ ID NO 11
 <211> LENGTH: 132
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis

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<400> SEQUENCE: 11

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Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly Phe
1           5           10           15
Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg Ser
20           25           30
Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly
35           40           45
Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val
50           55           60
Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val
65           70           75           80
Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala
85           90           95
Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp
100          105          110
Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu
115          120          125
Gly Pro Pro Ala
130

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<210> SEQ ID NO 12

<211> LENGTH: 195

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 12

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Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala Leu
1           5           10           15
Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Gly Pro Gln Val Val
20           25           30
Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly Thr
35           40           45
Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His Val
50           55           60
Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly Gln
65           70           75           80
Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val Ala
85           90           95
Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile Gly
100          105          110
Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly
115          120          125
Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu
130          135          140
Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu Thr
145          150          155          160
Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ser
165          170          175
Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr
180          185          190
Ala Ala Ser
195

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<210> SEQ ID NO 13
<211> LENGTH: 391
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 13

Met Val Asp Phe Gly Ala Leu Pro Pro Glu Ile Asn Ser Ala Arg Met
1      5      10      15

Tyr Ala Gly Pro Gly Ser Ala Ser Leu Val Ala Ala Ala Gln Met Trp
20     25     30

Asp Ser Val Ala Ser Asp Leu Phe Ser Ala Ala Ser Ala Phe Gln Ser
35     40     45

Val Val Trp Gly Leu Thr Val Gly Ser Trp Ile Gly Ser Ser Ala Gly
50     55     60

Leu Met Val Ala Ala Ala Ser Pro Tyr Val Ala Trp Met Ser Val Thr
65     70     75     80

Ala Gly Gln Ala Glu Leu Thr Ala Ala Gln Val Arg Val Ala Ala Ala
85     90     95

Ala Tyr Glu Thr Ala Tyr Gly Leu Thr Val Pro Pro Pro Val Ile Ala
100    105    110

Glu Asn Arg Ala Glu Leu Met Ile Leu Ile Ala Thr Asn Leu Leu Gly
115    120    125

Gln Asn Thr Pro Ala Ile Ala Val Asn Glu Ala Glu Tyr Gly Glu Met
130    135    140

Trp Ala Gln Asp Ala Ala Ala Met Phe Gly Tyr Ala Ala Ala Thr Ala
145    150    155    160

Thr Ala Thr Ala Thr Leu Leu Pro Phe Glu Glu Ala Pro Glu Met Thr
165    170    175

Ser Ala Gly Gly Leu Leu Glu Gln Ala Ala Ala Val Glu Glu Ala Ser
180    185    190

Asp Thr Ala Ala Ala Asn Gln Leu Met Asn Asn Val Pro Gln Ala Leu
195    200    205

Gln Gln Leu Ala Gln Pro Thr Gln Gly Thr Thr Pro Ser Ser Lys Leu
210    215    220

Gly Gly Leu Trp Lys Thr Val Ser Pro His Arg Ser Pro Ile Ser Asn
225    230    235    240

Met Val Ser Met Ala Asn Asn His Met Ser Met Thr Asn Ser Gly Val
245    250    255

Ser Met Thr Asn Thr Leu Ser Ser Met Leu Lys Gly Phe Ala Pro Ala
260    265    270

Ala Ala Ala Gln Ala Val Gln Thr Ala Ala Gln Asn Gly Val Arg Ala
275    280    285

Met Ser Ser Leu Gly Ser Ser Leu Gly Ser Ser Gly Leu Gly Gly Gly
290    295    300

Val Ala Ala Asn Leu Gly Arg Ala Ala Ser Val Gly Ser Leu Ser Val
305    310    315    320

Pro Gln Ala Trp Ala Ala Ala Asn Gln Ala Val Thr Pro Ala Ala Arg
325    330    335

Ala Leu Pro Leu Thr Ser Leu Thr Ser Ala Ala Glu Arg Gly Pro Gly
340    345    350

Gln Met Leu Gly Gly Leu Pro Val Gly Gln Met Gly Ala Arg Ala Gly
355    360    365

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Gly Gly Leu Ser Gly Val Leu Arg Val Pro Pro Arg Pro Tyr Val Met
 370 375 380

Pro His Ser Pro Ala Ala Gly
 385 390

<210> SEQ ID NO 14

<211> LENGTH: 392

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 14

Met Ser Arg Ala Phe Ile Ile Asp Pro Thr Ile Ser Ala Ile Asp Gly
 1 5 10 15

Leu Tyr Asp Leu Leu Gly Ile Gly Ile Pro Asn Gln Gly Gly Ile Leu
 20 25 30

Tyr Ser Ser Leu Glu Tyr Phe Glu Lys Ala Leu Glu Glu Leu Ala Ala
 35 40 45

Ala Phe Pro Gly Asp Gly Trp Leu Gly Ser Ala Ala Asp Lys Tyr Ala
 50 55 60

Gly Lys Asn Arg Asn His Val Asn Phe Phe Gln Glu Leu Ala Asp Leu
 65 70 75 80

Asp Arg Gln Leu Ile Ser Leu Ile His Asp Gln Ala Asn Ala Val Gln
 85 90 95

Thr Thr Arg Asp Ile Leu Glu Gly Ala Lys Lys Gly Leu Glu Phe Val
 100 105 110

Arg Pro Val Ala Val Asp Leu Thr Tyr Ile Pro Val Val Gly His Ala
 115 120 125

Leu Ser Ala Ala Phe Gln Ala Pro Phe Cys Ala Gly Ala Met Ala Val
 130 135 140

Val Gly Gly Ala Leu Ala Tyr Leu Val Val Lys Thr Leu Ile Asn Ala
 145 150 155 160

Thr Gln Leu Leu Lys Leu Leu Ala Lys Leu Ala Glu Leu Val Ala Ala
 165 170 175

Ala Ile Ala Asp Ile Ile Ser Asp Val Ala Asp Ile Ile Lys Gly Ile
 180 185 190

Leu Gly Glu Val Trp Glu Phe Ile Thr Asn Ala Leu Asn Gly Leu Lys
 195 200 205

Glu Leu Trp Asp Lys Leu Thr Gly Trp Val Thr Gly Leu Phe Ser Arg
 210 215 220

Gly Trp Ser Asn Leu Glu Ser Phe Phe Ala Gly Val Pro Gly Leu Thr
 225 230 235 240

Gly Ala Thr Ser Gly Leu Ser Gln Val Thr Gly Leu Phe Gly Ala Ala
 245 250 255

Gly Leu Ser Ala Ser Ser Gly Leu Ala His Ala Asp Ser Leu Ala Ser
 260 265 270

Ser Ala Ser Leu Pro Ala Leu Ala Gly Ile Gly Gly Gly Ser Gly Phe
 275 280 285

Gly Gly Leu Pro Ser Leu Ala Gln Val His Ala Ala Ser Thr Arg Gln
 290 295 300

Ala Leu Arg Pro Arg Ala Asp Gly Pro Val Gly Ala Ala Ala Glu Gln
 305 310 315 320

Val Gly Gly Gln Ser Gln Leu Val Ser Ala Gln Gly Ser Gln Gly Met

325										330					335				
Gly	Gly	Pro	Val	Gly	Met	Gly	Gly	Met	His	Pro	Ser	Ser	Gly	Ala	Ser				
			340				345						350						
Lys	Gly	Thr	Thr	Thr	Lys	Lys	Tyr	Ser	Glu	Gly	Ala	Ala	Ala	Gly	Thr				
			355				360						365						
Glu	Asp	Ala	Glu	Arg	Ala	Pro	Val	Glu	Ala	Asp	Ala	Gly	Gly	Gly	Gln				
			370				375						380						
Lys	Val	Leu	Val	Arg	Asn	Val	Val												
			385				390												
<210> SEQ ID NO 15																			
<211> LENGTH: 423																			
<212> TYPE: PRT																			
<213> ORGANISM: Mycobacterium tuberculosis																			
<400> SEQUENCE: 15																			
Met	Asp	Phe	Gly	Leu	Leu	Pro	Pro	Glu	Val	Asn	Ser	Ser	Arg	Met	Tyr				
1				5				10						15					
Ser	Gly	Pro	Gly	Pro	Glu	Ser	Met	Leu	Ala	Ala	Ala	Ala	Ala	Trp	Asp				
			20				25						30						
Gly	Val	Ala	Ala	Glu	Leu	Thr	Ser	Ala	Ala	Val	Ser	Tyr	Gly	Ser	Val				
			35				40						45						
Val	Ser	Thr	Leu	Ile	Val	Glu	Pro	Trp	Met	Gly	Pro	Ala	Ala	Ala	Ala				
			50				55						60						
Met	Ala	Ala	Ala	Ala	Thr	Pro	Tyr	Val	Gly	Trp	Leu	Ala	Ala	Thr	Ala				
65							70						75	80					
Ala	Leu	Ala	Lys	Glu	Thr	Ala	Thr	Gln	Ala	Arg	Ala	Ala	Ala	Glu	Ala				
			85				90						95						
Phe	Gly	Thr	Ala	Phe	Ala	Met	Thr	Val	Pro	Pro	Ser	Leu	Val	Ala	Ala				
			100				105						110						
Asn	Arg	Ser	Arg	Leu	Met	Ser	Leu	Val	Ala	Ala	Asn	Ile	Leu	Gly	Gln				
			115				120						125						
Asn	Ser	Ala	Ala	Ile	Ala	Ala	Thr	Gln	Ala	Glu	Tyr	Ala	Glu	Met	Trp				
			130				135						140						
Ala	Gln	Asp	Ala	Ala	Val	Met	Tyr	Ser	Tyr	Glu	Gly	Ala	Ser	Ala	Ala				
145				150						155			160						
Ala	Ser	Ala	Leu	Pro	Pro	Phe	Thr	Pro	Pro	Val	Gln	Gly	Thr	Gly	Pro				
			165				170						175						
Ala	Gly	Pro	Ala	Ala	Ala	Ala	Ala	Ala	Thr	Gln	Ala	Ala	Gly	Ala	Gly				
			180				185						190						
Ala	Val	Ala	Asp	Ala	Gln	Ala	Thr	Leu	Ala	Gln	Leu	Pro	Pro	Gly	Ile				
			195				200						205						
Leu	Ser	Asp	Ile	Leu	Ser	Ala	Leu	Ala	Ala	Asn	Ala	Asp	Pro	Leu	Thr				
			210				215						220						
Ser	Gly	Leu	Leu	Gly	Ile	Ala	Ser	Thr	Leu	Asn	Pro	Gln	Val	Gly	Ser				
225				230						235			240						
Ala	Gln	Pro	Ile	Val	Ile	Pro	Thr	Pro	Ile	Gly	Glu	Leu	Asp	Val	Ile				
			245				250						255						
Ala	Leu	Tyr	Ile	Ala	Ser	Ile	Ala	Thr	Gly	Ser	Ile	Ala	Leu	Ala	Ile				
			260				265						270						
Thr	Asn	Thr	Ala	Arg	Pro	Trp	His	Ile	Gly	Leu	Tyr	Gly	Asn	Ala	Gly				
			275				280						285						

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Gly Leu Gly Pro Thr Gln Gly His Pro Leu Ser Ser Ala Thr Asp Glu
 290 295 300

Pro Glu Pro His Trp Gly Pro Phe Gly Gly Ala Ala Pro Val Ser Ala
 305 310 315 320

Gly Val Gly His Ala Ala Leu Val Gly Ala Leu Ser Val Pro His Ser
 325 330 335

Trp Thr Thr Ala Ala Pro Glu Ile Gln Leu Ala Val Gln Ala Thr Pro
 340 345 350

Thr Phe Ser Ser Ser Ala Gly Ala Asp Pro Thr Ala Leu Asn Gly Met
 355 360 365

Pro Ala Gly Leu Leu Ser Gly Met Ala Leu Ala Ser Leu Ala Ala Arg
 370 375 380

Gly Thr Thr Gly Gly Gly Gly Thr Arg Ser Gly Thr Ser Thr Asp Gly
 385 390 395 400

Gln Glu Asp Gly Arg Lys Pro Pro Val Val Val Ile Arg Glu Gln Pro
 405 410 415

Pro Pro Gly Asn Pro Pro Arg
 420

<210> SEQ ID NO 16
 <211> LENGTH: 95
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis
 <220> FEATURE:
 <221> NAME/KEY: INIT_MET
 <222> LOCATION: (1)..(1)
 <220> FEATURE:
 <221> NAME/KEY: mat_peptide
 <222> LOCATION: (2)..(95)

<400> SEQUENCE: 16

Met Thr Glu Gln Gln Trp Asn Phe Ala Gly Ile Glu Ala Ala Ala Ser
 -1 1 5 10 15

Ala Ile Gln Gly Asn Val Thr Ser Ile His Ser Leu Leu Asp Glu Gly
 20 25 30

Lys Gln Ser Leu Thr Lys Leu Ala Ala Ala Trp Gly Gly Ser Gly Ser
 35 40 45

Glu Ala Tyr Gln Gly Val Gln Gln Lys Trp Asp Ala Thr Ala Thr Glu
 50 55 60

Leu Asn Asn Ala Leu Gln Asn Leu Ala Arg Thr Ile Ser Glu Ala Gly
 65 70 75

Gln Ala Met Ala Ser Thr Glu Gly Asn Val Thr Gly Met Phe Ala
 80 85 90

<210> SEQ ID NO 17
 <211> LENGTH: 338
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis
 <220> FEATURE:
 <221> NAME/KEY: mat_peptide
 <222> LOCATION: (43)..(338)

<400> SEQUENCE: 17

Met Gln Leu Val Asp Arg Val Arg Gly Ala Val Thr Gly Met Ser Arg
 -40 -35 -30 -25

Arg Leu Val Val Gly Ala Val Gly Ala Ala Leu Val Ser Gly Leu Val
 -20 -15 -10

-continued

Gly	Ala	Val	Gly	Gly	Thr	Ala	Thr	Ala	Gly	Ala	Phe	Ser	Arg	Pro	Gly
			-5				-1	1				5			
Leu	Pro	Val	Glu	Tyr	Leu	Gln	Val	Pro	Ser	Pro	Ser	Met	Gly	Arg	Asp
	10					15				20					
Ile	Lys	Val	Gln	Phe	Gln	Ser	Gly	Gly	Ala	Asn	Ser	Pro	Ala	Leu	Tyr
25					30					35				40	
Leu	Leu	Asp	Gly	Leu	Arg	Ala	Gln	Asp	Asp	Phe	Ser	Gly	Trp	Asp	Ile
			45						50					55	
Asn	Thr	Pro	Ala	Phe	Glu	Trp	Tyr	Asp	Gln	Ser	Gly	Leu	Ser	Val	Val
			60					65					70		
Met	Pro	Val	Gly	Gly	Gln	Ser	Ser	Phe	Tyr	Ser	Asp	Trp	Tyr	Gln	Pro
		75					80					85			
Ala	Cys	Gly	Lys	Ala	Gly	Cys	Gln	Thr	Tyr	Lys	Trp	Glu	Thr	Phe	Leu
	90					95					100				
Thr	Ser	Glu	Leu	Pro	Gly	Trp	Leu	Gln	Ala	Asn	Arg	His	Val	Lys	Pro
105					110					115				120	
Thr	Gly	Ser	Ala	Val	Val	Gly	Leu	Ser	Met	Ala	Ala	Ser	Ser	Ala	Leu
			125						130					135	
Thr	Leu	Ala	Ile	Tyr	His	Pro	Gln	Gln	Phe	Val	Tyr	Ala	Gly	Ala	Met
		140						145					150		
Ser	Gly	Leu	Leu	Asp	Pro	Ser	Gln	Ala	Met	Gly	Pro	Thr	Leu	Ile	Gly
		155					160					165			
Leu	Ala	Met	Gly	Asp	Ala	Gly	Gly	Tyr	Lys	Ala	Ser	Asp	Met	Trp	Gly
	170					175					180				
Pro	Lys	Glu	Asp	Pro	Ala	Trp	Gln	Arg	Asn	Asp	Pro	Leu	Leu	Asn	Val
185					190					195				200	
Gly	Lys	Leu	Ile	Ala	Asn	Asn	Thr	Arg	Val	Trp	Val	Tyr	Cys	Gly	Asn
			205						210					215	
Gly	Lys	Pro	Ser	Asp	Leu	Gly	Gly	Asn	Asn	Leu	Pro	Ala	Lys	Phe	Leu
		220						225					230		
Glu	Gly	Phe	Val	Arg	Thr	Ser	Asn	Ile	Lys	Phe	Gln	Asp	Ala	Tyr	Asn
		235					240					245			
Ala	Gly	Gly	Gly	His	Asn	Gly	Val	Phe	Asp	Phe	Pro	Asp	Ser	Gly	Thr
	250					255					260				
His	Ser	Trp	Glu	Tyr	Trp	Gly	Ala	Gln	Leu	Asn	Ala	Met	Lys	Pro	Asp
265					270					275				280	
Leu	Gln	Arg	Ala	Leu	Gly	Ala	Thr	Pro	Asn	Thr	Gly	Pro	Ala	Pro	Gln
			285						290					295	

Gly Ala

<210> SEQ ID NO 18
 <211> LENGTH: 325
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis
 <220> FEATURE:
 <221> NAME/KEY: mat_peptide
 <222> LOCATION: (41)..(325)

<400> SEQUENCE: 18

Met	Thr	Asp	Val	Ser	Arg	Lys	Ile	Arg	Ala	Trp	Gly	Arg	Arg	Leu	Met
-40					-35				-30					-25	
Ile	Gly	Thr	Ala	Ala	Ala	Val	Val	Leu	Pro	Gly	Leu	Val	Gly	Leu	Ala
			-20					-15						-10	

-continued

Gly Gly Ala Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly Leu Pro Val
 -5 -1 1 5
 Glu Tyr Leu Gln Val Pro Ser Pro Ser Met Gly Arg Asp Ile Lys Val
 10 15 20
 Gln Phe Gln Ser Gly Gly Asn Asn Ser Pro Ala Val Tyr Leu Leu Asp
 25 30 35 40
 Gly Leu Arg Ala Gln Asp Asp Tyr Asn Gly Trp Asp Ile Asn Thr Pro
 45 50 55
 Ala Phe Glu Trp Tyr Tyr Gln Ser Gly Leu Ser Ile Val Met Pro Val
 60 65 70
 Gly Gly Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Ser Pro Ala Cys Gly
 75 80 85
 Lys Ala Gly Cys Gln Thr Tyr Lys Trp Glu Thr Phe Leu Thr Ser Glu
 90 95 100
 Leu Pro Gln Trp Leu Ser Ala Asn Arg Ala Val Lys Pro Thr Gly Ser
 105 110 115 120
 Ala Ala Ile Gly Leu Ser Met Ala Gly Ser Ser Ala Met Ile Leu Ala
 125 130 135
 Ala Tyr His Pro Gln Gln Phe Ile Tyr Ala Gly Ser Leu Ser Ala Leu
 140 145 150
 Leu Asp Pro Ser Gln Gly Met Gly Pro Ser Leu Ile Gly Leu Ala Met
 155 160 165
 Gly Asp Ala Gly Gly Tyr Lys Ala Ala Asp Met Trp Gly Pro Ser Ser
 170 175 180
 Asp Pro Ala Trp Glu Arg Asn Asp Pro Thr Gln Gln Ile Pro Lys Leu
 185 190 195 200
 Val Ala Asn Asn Thr Arg Leu Trp Val Tyr Cys Gly Asn Gly Thr Pro
 205 210 215
 Asn Glu Leu Gly Gly Ala Asn Ile Pro Ala Glu Phe Leu Glu Asn Phe
 220 225 230
 Val Arg Ser Ser Asn Leu Lys Phe Gln Asp Ala Tyr Asn Ala Ala Gly
 235 240 245
 Gly His Asn Ala Val Phe Asn Phe Pro Pro Asn Gly Thr His Ser Trp
 250 255 260
 Glu Tyr Trp Gly Ala Gln Leu Asn Ala Met Lys Gly Asp Leu Gln Ser
 265 270 275 280
 Ser Leu Gly Ala Gly
 285

<210> SEQ ID NO 19
 <211> LENGTH: 144
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis
 <220> FEATURE:
 <221> NAME/KEY: INIT_MET
 <222> LOCATION: (1)..(1)
 <220> FEATURE:
 <221> NAME/KEY: mat_peptide
 <222> LOCATION: (2)..(144)

<400> SEQUENCE: 19

Met Ala Thr Thr Leu Pro Val Gln Arg His Pro Arg Ser Leu Phe Pro
 -1 1 5 10 15
 Glu Phe Ser Glu Leu Phe Ala Ala Phe Pro Ser Phe Ala Gly Leu Arg

-continued

20					25					30					
Pro	Thr	Phe	Asp	Thr	Arg	Leu	Met	Arg	Leu	Glu	Asp	Glu	Met	Lys	Glu
		35					40					45			
Gly	Arg	Tyr	Glu	Val	Arg	Ala	Glu	Leu	Pro	Gly	Val	Asp	Pro	Asp	Lys
		50					55					60			
Asp	Val	Asp	Ile	Met	Val	Arg	Asp	Gly	Gln	Leu	Thr	Ile	Lys	Ala	Glu
		65					70					75			80
Arg	Thr	Glu	Gln	Lys	Asp	Phe	Asp	Gly	Arg	Ser	Glu	Phe	Ala	Tyr	Gly
				85					90						95
Ser	Phe	Val	Arg	Thr	Val	Ser	Leu	Pro	Val	Gly	Ala	Asp	Glu	Asp	Asp
				100					105						110
Ile	Lys	Ala	Thr	Tyr	Asp	Lys	Gly	Ile	Leu	Thr	Val	Ser	Val	Ala	Val
				115					120						125
Ser	Glu	Gly	Lys	Pro	Thr	Glu	Lys	His	Ile	Gln	Ile	Arg	Ser	Thr	Asn
				130					135						140

<210> SEQ ID NO 20

<211> LENGTH: 228

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<220> FEATURE:

<221> NAME/KEY: mat_peptide

<222> LOCATION: (24)..(228)

<400> SEQUENCE: 20

Met	Arg	Ile	Lys	Ile	Phe	Met	Leu	Val	Thr	Ala	Val	Val	Leu	Leu	Cys
-20					-15							-10			-5
Cys	Ser	Gly	Val	Ala	Thr	Ala	Ala	Pro	Lys	Thr	Tyr	Cys	Glu	Glu	Leu
		-1	1					5					10		
Lys	Gly	Thr	Asp	Thr	Gly	Gln	Ala	Cys	Gln	Ile	Gln	Met	Ser	Asp	Pro
		15					20					25			
Ala	Tyr	Asn	Ile	Asn	Ile	Ser	Leu	Pro	Ser	Tyr	Tyr	Pro	Asp	Gln	Lys
		30					35					40			
Ser	Leu	Glu	Asn	Tyr	Ile	Ala	Gln	Thr	Arg	Asp	Lys	Phe	Leu	Ser	Ala
		45					50					55			60
Ala	Thr	Ser	Ser	Thr	Pro	Arg	Glu	Ala	Pro	Tyr	Glu	Leu	Asn	Ile	Thr
				65					70						75
Ser	Ala	Thr	Tyr	Gln	Ser	Ala	Ile	Pro	Pro	Arg	Gly	Thr	Gln	Ala	Val
			80					85					90		
Val	Leu	Lys	Val	Tyr	Gln	Asn	Ala	Gly	Gly	Thr	His	Pro	Thr	Thr	Thr
		95					100					105			
Tyr	Lys	Ala	Phe	Asp	Trp	Asp	Gln	Ala	Tyr	Arg	Lys	Pro	Ile	Thr	Tyr
		110					115					120			
Asp	Thr	Leu	Trp	Gln	Ala	Asp	Thr	Asp	Pro	Leu	Pro	Val	Val	Phe	Pro
		125					130					135			140
Ile	Val	Gln	Gly	Glu	Leu	Ser	Lys	Gln	Thr	Gly	Gln	Gln	Val	Ser	Ile
				145					150					155	
Ala	Pro	Asn	Ala	Gly	Leu	Asp	Pro	Val	Asn	Tyr	Gln	Asn	Phe	Ala	Val
			160					165					170		
Thr	Asn	Asp	Gly	Val	Ile	Phe	Phe	Phe	Asn	Pro	Gly	Glu	Leu	Leu	Pro
			175					180				185			
Glu	Ala	Ala	Gly	Pro	Thr	Gln	Val	Leu	Val	Pro	Arg	Ser	Ala	Ile	Asp
							195								200

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Ser Met Leu Ala
205

<210> SEQ ID NO 21
 <211> LENGTH: 355
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis
 <220> FEATURE:
 <221> NAME/KEY: mat_peptide
 <222> LOCATION: (33) .. (355)

<400> SEQUENCE: 21

Met Ser Asn Ser Arg Arg Arg Ser Leu Arg Trp Ser Trp Leu Leu Ser
 -30 -25 -20 -15
 Val Leu Ala Ala Val Gly Leu Gly Leu Ala Thr Ala Pro Ala Gln Ala
 -10 -5 -1 1
 Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala Leu
 5 10 15
 Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Gly Pro Gln Val Val
 20 25 30
 Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly Thr
 35 40 45 50
 Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His Val
 55 60 65
 Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly Gln
 70 75 80
 Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val Ala
 85 90 95
 Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile Gly
 100 105 110
 Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly
 115 120 125 130
 Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu
 135 140 145
 Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu Thr
 150 155 160
 Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ser
 165 170 175
 Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr
 180 185 190
 Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly Phe Ala
 195 200 205 210
 Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg Ser Gly
 215 220 225
 Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly Leu
 230 235 240
 Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val Val
 245 250 255
 Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val Ile
 260 265 270
 Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala Asp
 275 280 285 290
 Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp Gln
 295 300 305

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Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu Gly
 310 315 320

Pro Pro Ala

<210> SEQ ID NO 22
 <211> LENGTH: 323
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Ser/Ala mutant of mature Mtb32A

<400> SEQUENCE: 22

Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala Leu
 1 5 10 15

Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Gly Pro Gln Val Val
 20 25 30

Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly Thr
 35 40 45

Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His Val
 50 55 60

Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly Gln
 65 70 75 80

Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val Ala
 85 90 95

Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile Gly
 100 105 110

Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly
 115 120 125

Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu
 130 135 140

Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu Thr
 145 150 155 160

Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ala
 165 170 175

Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr
 180 185 190

Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly Phe Ala
 195 200 205

Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg Ser Gly
 210 215 220

Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly Leu
 225 230 235 240

Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val Val
 245 250 255

Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val Ile
 260 265 270

Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala Asp
 275 280 285

Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp Gln
 290 295 300

Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu Gly
 305 310 315 320

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Pro Pro Ala

<210> SEQ ID NO 23
 <211> LENGTH: 96
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 23

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Met Ser Gln Ile Met Tyr Asn Tyr Pro Ala Met Leu Gly His Ala Gly
1           5           10           15
Asp Met Ala Gly Tyr Ala Gly Thr Leu Gln Ser Leu Gly Ala Glu Ile
          20           25           30
Ala Val Glu Gln Ala Ala Leu Gln Ser Ala Trp Gln Gly Asp Thr Gly
          35           40           45
Ile Thr Tyr Gln Ala Trp Gln Ala Gln Trp Asn Gln Ala Met Glu Asp
          50           55           60
Leu Val Arg Ala Tyr His Ala Met Ser Ser Thr His Glu Ala Asn Thr
65           70           75           80
Met Ala Met Met Ala Arg Asp Thr Ala Glu Ala Ala Lys Trp Gly Gly
          85           90           95

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<210> SEQ ID NO 24
 <211> LENGTH: 723
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Mtb72f

<400> SEQUENCE: 24

```

Met Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly
1           5           10           15
Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg
          20           25           30
Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu
          35           40           45
Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg
          50           55           60
Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp
65           70           75           80
Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met
          85           90           95
Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr
          100          105          110
Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala
          115          120          125
Glu Gly Pro Pro Ala Glu Phe Met Val Asp Phe Gly Ala Leu Pro Pro
          130          135          140
Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly Pro Gly Ser Ala Ser Leu
145          150          155          160
Val Ala Ala Ala Gln Met Trp Asp Ser Val Ala Ser Asp Leu Phe Ser
          165          170          175
Ala Ala Ser Ala Phe Gln Ser Val Val Trp Gly Leu Thr Val Gly Ser
          180          185          190
Trp Ile Gly Ser Ser Ala Gly Leu Met Val Ala Ala Ala Ser Pro Tyr
          195          200          205

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Val	Ala	Trp	Met	Ser	Val	Thr	Ala	Gly	Gln	Ala	Glu	Leu	Thr	Ala	Ala	210	215	220	
Gln	Val	Arg	Val	Ala	Ala	Ala	Ala	Tyr	Glu	Thr	Ala	Tyr	Gly	Leu	Thr	225	230	235	240
Val	Pro	Pro	Pro	Val	Ile	Ala	Glu	Asn	Arg	Ala	Glu	Leu	Met	Ile	Leu	245	250	255	
Ile	Ala	Thr	Asn	Leu	Leu	Gly	Gln	Asn	Thr	Pro	Ala	Ile	Ala	Val	Asn	260	265	270	
Glu	Ala	Glu	Tyr	Gly	Glu	Met	Trp	Ala	Gln	Asp	Ala	Ala	Ala	Met	Phe	275	280	285	
Gly	Tyr	Ala	Ala	Ala	Thr	Ala	Thr	Ala	Thr	Ala	Thr	Leu	Leu	Pro	Phe	290	295	300	
Glu	Glu	Ala	Pro	Glu	Met	Thr	Ser	Ala	Gly	Gly	Leu	Leu	Glu	Gln	Ala	305	310	315	320
Ala	Ala	Val	Glu	Glu	Ala	Ser	Asp	Thr	Ala	Ala	Ala	Asn	Gln	Leu	Met	325	330	335	
Asn	Asn	Val	Pro	Gln	Ala	Leu	Gln	Gln	Leu	Ala	Gln	Pro	Thr	Gln	Gly	340	345	350	
Thr	Thr	Pro	Ser	Ser	Lys	Leu	Gly	Gly	Leu	Trp	Lys	Thr	Val	Ser	Pro	355	360	365	
His	Arg	Ser	Pro	Ile	Ser	Asn	Met	Val	Ser	Met	Ala	Asn	Asn	His	Met	370	375	380	
Ser	Met	Thr	Asn	Ser	Gly	Val	Ser	Met	Thr	Asn	Thr	Leu	Ser	Ser	Met	385	390	395	400
Leu	Lys	Gly	Phe	Ala	Pro	Ala	Ala	Ala	Ala	Gln	Ala	Val	Gln	Thr	Ala	405	410	415	
Ala	Gln	Asn	Gly	Val	Arg	Ala	Met	Ser	Ser	Leu	Gly	Ser	Ser	Leu	Gly	420	425	430	
Ser	Ser	Gly	Leu	Gly	Gly	Gly	Val	Ala	Ala	Asn	Leu	Gly	Arg	Ala	Ala	435	440	445	
Ser	Val	Gly	Ser	Leu	Ser	Val	Pro	Gln	Ala	Trp	Ala	Ala	Ala	Asn	Gln	450	455	460	
Ala	Val	Thr	Pro	Ala	Ala	Arg	Ala	Leu	Pro	Leu	Thr	Ser	Leu	Thr	Ser	465	470	475	480
Ala	Ala	Glu	Arg	Gly	Pro	Gly	Gln	Met	Leu	Gly	Gly	Leu	Pro	Val	Gly	485	490	495	
Gln	Met	Gly	Ala	Arg	Ala	Gly	Gly	Gly	Leu	Ser	Gly	Val	Leu	Arg	Val	500	505	510	
Pro	Pro	Arg	Pro	Tyr	Val	Met	Pro	His	Ser	Pro	Ala	Ala	Gly	Asp	Ile	515	520	525	
Ala	Pro	Pro	Ala	Leu	Ser	Gln	Asp	Arg	Phe	Ala	Asp	Phe	Pro	Ala	Leu	530	535	540	
Pro	Leu	Asp	Pro	Ser	Ala	Met	Val	Ala	Gln	Val	Gly	Pro	Gln	Val	Val	545	550	555	560
Asn	Ile	Asn	Thr	Lys	Leu	Gly	Tyr	Asn	Asn	Ala	Val	Gly	Ala	Gly	Thr	565	570	575	
Gly	Ile	Val	Ile	Asp	Pro	Asn	Gly	Val	Val	Leu	Thr	Asn	Asn	His	Val	580	585	590	
Ile	Ala	Gly	Ala	Thr	Asp	Ile	Asn	Ala	Phe	Ser	Val	Gly	Ser	Gly	Gln	595	600	605	

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Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val Ala
 610 615 620
 Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile Gly
 625 630 635 640
 Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly
 645 650 655
 Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu
 660 665 670
 Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu Thr
 675 680 685
 Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ser
 690 695 700
 Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr
 705 710 715 720
 Ala Ala Ser

<210> SEQ ID NO 25
 <211> LENGTH: 723
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: M72

<400> SEQUENCE: 25

Met Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly
 1 5 10 15
 Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg
 20 25 30
 Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu
 35 40 45
 Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg
 50 55 60
 Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp
 65 70 75 80
 Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met
 85 90 95
 Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr
 100 105 110
 Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala
 115 120 125
 Glu Gly Pro Pro Ala Glu Phe Met Val Asp Phe Gly Ala Leu Pro Pro
 130 135 140
 Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly Pro Gly Ser Ala Ser Leu
 145 150 155 160
 Val Ala Ala Ala Gln Met Trp Asp Ser Val Ala Ser Asp Leu Phe Ser
 165 170 175
 Ala Ala Ser Ala Phe Gln Ser Val Val Trp Gly Leu Thr Val Gly Ser
 180 185 190
 Trp Ile Gly Ser Ser Ala Gly Leu Met Val Ala Ala Ala Ser Pro Tyr
 195 200 205
 Val Ala Trp Met Ser Val Thr Ala Gly Gln Ala Glu Leu Thr Ala Ala
 210 215 220
 Gln Val Arg Val Ala Ala Ala Ala Tyr Glu Thr Ala Tyr Gly Leu Thr

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225					230					235					240
Val	Pro	Pro	Pro	Val	Ile	Ala	Glu	Asn	Arg	Ala	Glu	Leu	Met	Ile	Leu
				245					250					255	
Ile	Ala	Thr	Asn	Leu	Leu	Gly	Gln	Asn	Thr	Pro	Ala	Ile	Ala	Val	Asn
			260					265					270		
Glu	Ala	Glu	Tyr	Gly	Glu	Met	Trp	Ala	Gln	Asp	Ala	Ala	Ala	Met	Phe
		275					280					285			
Gly	Tyr	Ala	Ala	Ala	Thr	Ala	Thr	Ala	Thr	Ala	Thr	Leu	Leu	Pro	Phe
	290					295					300				
Glu	Glu	Ala	Pro	Glu	Met	Thr	Ser	Ala	Gly	Gly	Leu	Leu	Glu	Gln	Ala
305					310				315					320	
Ala	Ala	Val	Glu	Glu	Ala	Ser	Asp	Thr	Ala	Ala	Ala	Asn	Gln	Leu	Met
				325					330					335	
Asn	Asn	Val	Pro	Gln	Ala	Leu	Gln	Gln	Leu	Ala	Gln	Pro	Thr	Gln	Gly
			340					345					350		
Thr	Thr	Pro	Ser	Ser	Lys	Leu	Gly	Gly	Leu	Trp	Lys	Thr	Val	Ser	Pro
		355					360					365			
His	Arg	Ser	Pro	Ile	Ser	Asn	Met	Val	Ser	Met	Ala	Asn	Asn	His	Met
	370					375					380				
Ser	Met	Thr	Asn	Ser	Gly	Val	Ser	Met	Thr	Asn	Thr	Leu	Ser	Ser	Met
385					390					395				400	
Leu	Lys	Gly	Phe	Ala	Pro	Ala	Ala	Ala	Ala	Gln	Ala	Val	Gln	Thr	Ala
				405					410					415	
Ala	Gln	Asn	Gly	Val	Arg	Ala	Met	Ser	Ser	Leu	Gly	Ser	Ser	Leu	Gly
			420					425					430		
Ser	Ser	Gly	Leu	Gly	Gly	Val	Ala	Ala	Asn	Leu	Gly	Arg	Ala	Ala	
		435				440					445				
Ser	Val	Gly	Ser	Leu	Ser	Val	Pro	Gln	Ala	Trp	Ala	Ala	Ala	Asn	Gln
	450					455					460				
Ala	Val	Thr	Pro	Ala	Ala	Arg	Ala	Leu	Pro	Leu	Thr	Ser	Leu	Thr	Ser
465					470					475				480	
Ala	Ala	Glu	Arg	Gly	Pro	Gly	Gln	Met	Leu	Gly	Gly	Leu	Pro	Val	Gly
				485					490					495	
Gln	Met	Gly	Ala	Arg	Ala	Gly	Gly	Gly	Leu	Ser	Gly	Val	Leu	Arg	Val
			500					505					510		
Pro	Pro	Arg	Pro	Tyr	Val	Met	Pro	His	Ser	Pro	Ala	Ala	Gly	Asp	Ile
		515					520					525			
Ala	Pro	Pro	Ala	Leu	Ser	Gln	Asp	Arg	Phe	Ala	Asp	Phe	Pro	Ala	Leu
	530					535					540				
Pro	Leu	Asp	Pro	Ser	Ala	Met	Val	Ala	Gln	Val	Gly	Pro	Gln	Val	Val
545					550					555				560	
Asn	Ile	Asn	Thr	Lys	Leu	Gly	Tyr	Asn	Asn	Ala	Val	Gly	Ala	Gly	Thr
				565				570						575	
Gly	Ile	Val	Ile	Asp	Pro	Asn	Gly	Val	Val	Leu	Thr	Asn	Asn	His	Val
			580					585					590		
Ile	Ala	Gly	Ala	Thr	Asp	Ile	Asn	Ala	Phe	Ser	Val	Gly	Ser	Gly	Gln
		595					600					605			
Thr	Tyr	Gly	Val	Asp	Val	Val	Gly	Tyr	Asp	Arg	Thr	Gln	Asp	Val	Ala
	610					615					620				
Val	Leu	Gln	Leu	Arg	Gly	Ala	Gly	Gly	Leu	Pro	Ser	Ala	Ala	Ile	Gly
625					630					635				640	

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Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly
645 650 655

Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu
660 665 670

Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu Thr
675 680 685

Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ala
690 695 700

Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr
705 710 715 720

Ala Ala Ser

<210> SEQ ID NO 26
<211> LENGTH: 702
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Mtb71f

<400> SEQUENCE: 26

Asp Pro Val Asp Ala Val Ile Asn Thr Thr Cys Asn Tyr Gly Gln Val
1 5 10 15

Val Ala Ala Leu Asn Ala Thr Asp Pro Gly Ala Ala Ala Gln Phe Asn
20 25 30

Ala Ser Pro Val Ala Gln Ser Tyr Leu Arg Asn Phe Leu Ala Ala Pro
35 40 45

Pro Pro Gln Arg Ala Ala Met Ala Ala Gln Leu Gln Ala Val Pro Gly
50 55 60

Ala Ala Gln Tyr Ile Gly Leu Val Glu Ser Val Ala Gly Ser Cys Asn
65 70 75 80

Asn Tyr Glu Leu Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp Ala
85 90 95

His Gly Ala Met Ile Arg Ala Gln Ala Ala Ser Leu Glu Ala Glu His
100 105 110

Gln Ala Ile Val Arg Asp Val Leu Ala Ala Gly Asp Phe Trp Gly Gly
115 120 125

Ala Gly Ser Val Ala Cys Gln Glu Phe Ile Thr Gln Leu Gly Arg Asn
130 135 140

Phe Gln Val Ile Tyr Glu Gln Ala Asn Ala His Gly Gln Lys Val Gln
145 150 155 160

Ala Ala Gly Asn Asn Met Ala Gln Thr Asp Ser Ala Val Gly Ser Ser
165 170 175

Trp Ala Thr Ser Met Ser Leu Leu Asp Ala His Ile Pro Gln Leu Val
180 185 190

Ala Ser Gln Ser Ala Phe Ala Ala Lys Ala Gly Leu Met Arg His Thr
195 200 205

Ile Gly Gln Ala Glu Gln Ala Ala Met Ser Ala Gln Ala Phe His Gln
210 215 220

Gly Glu Ser Ser Ala Ala Phe Gln Ala Ala His Ala Arg Phe Val Ala
225 230 235 240

Ala Ala Ala Lys Val Asn Thr Leu Leu Asp Val Ala Gln Ala Asn Leu
245 250 255

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Gly	Glu	Ala	Ala	Gly	Thr	Tyr	Val	Ala	Ala	Asp	Ala	Ala	Ala	Ala	Ser
		260						265					270		
Thr	Tyr	Thr	Gly	Phe	Asp	Ile	Met	Asp	Phe	Gly	Leu	Leu	Pro	Pro	Glu
		275					280					285			
Val	Asn	Ser	Ser	Arg	Met	Tyr	Ser	Gly	Pro	Gly	Pro	Glu	Ser	Met	Leu
	290					295					300				
Ala	Ala	Ala	Ala	Ala	Trp	Asp	Gly	Val	Ala	Ala	Glu	Leu	Thr	Ser	Ala
305					310					315					320
Ala	Val	Ser	Tyr	Gly	Ser	Val	Val	Ser	Thr	Leu	Ile	Val	Glu	Pro	Trp
				325					330					335	
Met	Gly	Pro	Ala	Ala	Ala	Ala	Met	Ala	Ala	Ala	Ala	Thr	Pro	Tyr	Val
			340					345					350		
Gly	Trp	Leu	Ala	Ala	Thr	Ala	Ala	Leu	Ala	Lys	Glu	Thr	Ala	Thr	Gln
		355					360					365			
Ala	Arg	Ala	Ala	Ala	Glu	Ala	Phe	Gly	Thr	Ala	Phe	Ala	Met	Thr	Val
	370					375				380					
Pro	Pro	Ser	Leu	Val	Ala	Ala	Asn	Arg	Ser	Arg	Leu	Met	Ser	Leu	Val
385					390					395					400
Ala	Ala	Asn	Ile	Leu	Gly	Gln	Asn	Ser	Ala	Ala	Ile	Ala	Ala	Thr	Gln
			405						410					415	
Ala	Glu	Tyr	Ala	Glu	Met	Trp	Ala	Gln	Asp	Ala	Ala	Val	Met	Tyr	Ser
			420					425					430		
Tyr	Glu	Gly	Ala	Ser	Ala	Ala	Ala	Ser	Ala	Leu	Pro	Pro	Phe	Thr	Pro
		435					440					445			
Pro	Val	Gln	Gly	Thr	Gly	Pro	Ala	Gly	Pro	Ala	Ala	Ala	Ala	Ala	Ala
	450					455				460					
Thr	Gln	Ala	Ala	Gly	Ala	Gly	Ala	Val	Ala	Asp	Ala	Gln	Ala	Thr	Leu
465					470					475					480
Ala	Gln	Leu	Pro	Pro	Gly	Ile	Leu	Ser	Asp	Ile	Leu	Ser	Ala	Leu	Ala
				485					490					495	
Ala	Asn	Ala	Asp	Pro	Leu	Thr	Ser	Gly	Leu	Leu	Gly	Ile	Ala	Ser	Thr
			500					505					510		
Leu	Asn	Pro	Gln	Val	Gly	Ser	Ala	Gln	Pro	Ile	Val	Ile	Pro	Thr	Pro
		515					520					525			
Ile	Gly	Glu	Leu	Asp	Val	Ile	Ala	Leu	Tyr	Ile	Ala	Ser	Ile	Ala	Thr
	530					535					540				
Gly	Ser	Ile	Ala	Leu	Ala	Ile	Thr	Asn	Thr	Ala	Arg	Pro	Trp	His	Ile
545					550					555					560
Gly	Leu	Tyr	Gly	Asn	Ala	Gly	Gly	Leu	Gly	Pro	Thr	Gln	Gly	His	Pro
				565					570					575	
Leu	Ser	Ser	Ala	Thr	Asp	Glu	Pro	Glu	Pro	His	Trp	Gly	Pro	Phe	Gly
			580					585					590		
Gly	Ala	Ala	Pro	Val	Ser	Ala	Gly	Val	Gly	His	Ala	Ala	Leu	Val	Gly
		595					600					605			
Ala	Leu	Ser	Val	Pro	His	Ser	Trp	Thr	Thr	Ala	Ala	Pro	Glu	Ile	Gln
	610					615						620			
Leu	Ala	Val	Gln	Ala	Thr	Pro	Thr	Phe	Ser	Ser	Ser	Ala	Gly	Ala	Asp
625					630					635					640
Pro	Thr	Ala	Leu	Asn	Gly	Met	Pro	Ala	Gly	Leu	Leu	Ser	Gly	Met	Ala
				645					650					655	
Leu	Ala	Ser	Leu	Ala	Ala	Arg	Gly	Thr	Thr	Gly	Gly	Gly	Gly	Thr	Arg

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660	665	670
Ser Gly Thr	Ser Thr Asp Gly Gln Glu Asp Gly Arg Lys Pro Pro Val	
675	680	685
Val Val Ile Arg Glu Gln Pro Pro Pro Gly Asn Pro Pro Arg		
690	695	700

<210> SEQ ID NO 27
 <211> LENGTH: 920
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: M72-Mtb9.9-Mtb9.8
 <400> SEQUENCE: 27

Met Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly	
1 5 10 15	
Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg	
20 25 30	
Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu	
35 40 45	
Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg	
50 55 60	
Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp	
65 70 75 80	
Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met	
85 90 95	
Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr	
100 105 110	
Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala	
115 120 125	
Glu Gly Pro Pro Ala Glu Phe Met Val Asp Phe Gly Ala Leu Pro Pro	
130 135 140	
Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly Pro Gly Ser Ala Ser Leu	
145 150 155 160	
Val Ala Ala Ala Gln Met Trp Asp Ser Val Ala Ser Asp Leu Phe Ser	
165 170 175	
Ala Ala Ser Ala Phe Gln Ser Val Val Trp Gly Leu Thr Val Gly Ser	
180 185 190	
Trp Ile Gly Ser Ser Ala Gly Leu Met Val Ala Ala Ala Ser Pro Tyr	
195 200 205	
Val Ala Trp Met Ser Val Thr Ala Gly Gln Ala Glu Leu Thr Ala Ala	
210 215 220	
Gln Val Arg Val Ala Ala Ala Tyr Glu Thr Ala Tyr Gly Leu Thr	
225 230 235 240	
Val Pro Pro Pro Val Ile Ala Glu Asn Arg Ala Glu Leu Met Ile Leu	
245 250 255	
Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr Pro Ala Ile Ala Val Asn	
260 265 270	
Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln Asp Ala Ala Ala Met Phe	
275 280 285	
Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr Leu Leu Pro Phe	
290 295 300	
Glu Glu Ala Pro Glu Met Thr Ser Ala Gly Gly Leu Leu Glu Gln Ala	

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305	310	315	320
Ala Ala Val Glu Glu 325	Ala Ser Asp Thr 330	Ala Ala Asn Gln 335	Leu Met
Asn Asn Val Pro Gln 340	Ala Leu Gln Gln 345	Ala Gln Pro Thr 350	Gln Gly
Thr Thr Pro Ser Ser 355	Lys Leu Gly Gly 360	Leu Trp Lys Thr 365	Val Ser Pro
His Arg Ser Pro Ile 370	Ser Asn Met Val 375	Ser Met Ala Asn 380	Asn His Met
Ser Met Thr Asn Ser 385	Gly Val Ser Met 390	Thr Asn Thr Leu 395	Ser Ser Met 400
Leu Lys Gly Phe Ala 405	Pro Ala Ala Ala 410	Gln Ala Val Gln 415	Thr Ala
Ala Gln Asn Gly Val 420	Arg Ala Met Ser 425	Ser Leu Gly Ser 430	Ser Leu Gly
Ser Ser Gly Leu Gly 435	Gly Gly Val Ala 440	Ala Asn Leu Gly 445	Arg Ala Ala
Ser Val Gly Ser Leu 450	Ser Val Pro Gln 455	Ala Trp Ala Ala 460	Ala Asn Gln
Ala Val Thr Pro Ala 465	Ala Arg Ala Leu 470	Pro Leu Thr Ser 475	Leu Thr Ser 480
Ala Ala Glu Arg Gly 485	Pro Gly Gln Met 490	Leu Gly Gly Leu 495	Pro Val Gly 500
Gln Met Gly Ala Arg 500	Ala Gly Gly Gly 505	Leu Ser Gly Val 510	Leu Arg Val
Pro Pro Arg Pro Tyr 515	Val Met Pro His 520	Ser Pro Ala Ala 525	Gly Asp Ile
Ala Pro Pro Ala Leu 530	Ser Gln Asp Arg 535	Phe Ala Asp Phe 540	Pro Ala Leu
Pro Leu Asp Pro Ser 545	Ala Met Val Ala 550	Gln Val Gly Pro 555	Gln Val Val 560
Asn Ile Asn Thr Lys 565	Leu Gly Tyr Asn 570	Asn Ala Val Gly 575	Ala Gly Thr 580
Gly Ile Val Ile Asp 580	Pro Asn Gly Val 585	Val Leu Thr Asn 590	Asn His Val
Ile Ala Gly Ala Thr 595	Asp Ile Asn Ala 600	Phe Ser Val Gly 605	Ser Gly Gln
Thr Tyr Gly Val Asp 610	Val Val Gly Tyr 615	Asp Arg Thr Gln 620	Asp Val Ala
Val Leu Gln Leu Arg 625	Gly Ala Gly Gly 630	Leu Pro Ser Ala 635	Ala Ile Gly 640
Gly Gly Val Ala Val 645	Gly Glu Pro Val 650	Ala Met Gly Asn 655	Ser Gly
Gly Gln Gly Gly Thr 660	Pro Arg Ala Val 665	Pro Gly Arg Val 670	Val Ala Leu
Gly Gln Thr Val Gln 675	Ala Ser Asp Ser 680	Leu Thr Gly Ala 685	Glu Glu Thr
Leu Asn Gly Leu Ile 690	Gln Phe Asp Ala 695	Ala Ala Ile Gln 700	Pro Gly Asp Ala
Gly Gly Pro Val Val 705	Asn Gly Leu Gly 710	Gln Val Val Gly 715	Met Asn Thr 720

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Ala Ala Ser Ser Thr Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp
725 730 735

Ala His Gly Ala Met Ile Arg Ala Gln Ala Ala Ser Leu Glu Ala Glu
740 745 750

His Gln Ala Ile Val Arg Asp Val Leu Ala Ala Gly Asp Phe Trp Gly
755 760 765

Gly Ala Gly Ser Val Ala Cys Gln Glu Phe Ile Thr Gln Leu Gly Arg
770 775 780

Asn Phe Gln Val Ile Tyr Glu Gln Ala Asn Ala His Gly Gln Lys Val
785 790 795 800

Gln Ala Ala Gly Asn Asn Met Ala Gln Thr Asp Ser Ala Val Gly Ser
805 810 815

Ser Trp Ala Thr Ser Met Ser Leu Leu Asp Ala His Ile Pro Gln Leu
820 825 830

Val Ala Ser Gln Ser Ala Phe Ala Ala Lys Ala Gly Leu Met Arg His
835 840 845

Thr Ile Gly Gln Ala Glu Gln Ala Ala Met Ser Ala Gln Ala Phe His
850 855 860

Gln Gly Glu Ser Ser Ala Ala Phe Gln Ala Ala His Ala Arg Phe Val
865 870 875 880

Ala Ala Ala Ala Lys Val Asn Thr Leu Leu Asp Val Ala Gln Ala Asn
885 890 895

Leu Gly Glu Ala Ala Gly Thr Tyr Val Ala Ala Asp Ala Ala Ala
900 905 910

Ser Thr Tyr Thr Gly Phe Pro Trp
915 920

<210> SEQ ID NO 28
<211> LENGTH: 1010
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: M103

<400> SEQUENCE: 28

Met Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly
1 5 10 15

Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg
20 25 30

Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu
35 40 45

Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg
50 55 60

Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp
65 70 75 80

Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met
85 90 95

Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr
100 105 110

Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala
115 120 125

Glu Gly Pro Pro Ala Glu Phe Met Val Asp Phe Gly Ala Leu Pro Pro
130 135 140

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Glu	Ile	Asn	Ser	Ala	Arg	Met	Tyr	Ala	Gly	Pro	Gly	Ser	Ala	Ser	Leu	145	150	155	160
Val	Ala	Ala	Ala	Gln	Met	Trp	Asp	Ser	Val	Ala	Ser	Asp	Leu	Phe	Ser	165	170	175	
Ala	Ala	Ser	Ala	Phe	Gln	Ser	Val	Val	Trp	Gly	Leu	Thr	Val	Gly	Ser	180	185	190	
Trp	Ile	Gly	Ser	Ser	Ala	Gly	Leu	Met	Val	Ala	Ala	Ala	Ser	Pro	Tyr	195	200	205	
Val	Ala	Trp	Met	Ser	Val	Thr	Ala	Gly	Gln	Ala	Glu	Leu	Thr	Ala	Ala	210	215	220	
Gln	Val	Arg	Val	Ala	Ala	Ala	Tyr	Glu	Thr	Ala	Tyr	Gly	Leu	Thr		225	230	235	240
Val	Pro	Pro	Pro	Val	Ile	Ala	Glu	Asn	Arg	Ala	Glu	Leu	Met	Ile	Leu	245	250	255	
Ile	Ala	Thr	Asn	Leu	Leu	Gly	Gln	Asn	Thr	Pro	Ala	Ile	Ala	Val	Asn	260	265	270	
Glu	Ala	Glu	Tyr	Gly	Glu	Met	Trp	Ala	Gln	Asp	Ala	Ala	Ala	Met	Phe	275	280	285	
Gly	Tyr	Ala	Ala	Ala	Thr	Ala	Thr	Ala	Thr	Ala	Thr	Leu	Leu	Pro	Phe	290	295	300	
Glu	Glu	Ala	Pro	Glu	Met	Thr	Ser	Ala	Gly	Gly	Leu	Leu	Glu	Gln	Ala	305	310	315	320
Ala	Ala	Val	Glu	Glu	Ala	Ser	Asp	Thr	Ala	Ala	Ala	Asn	Gln	Leu	Met	325	330	335	
Asn	Asn	Val	Pro	Gln	Ala	Leu	Gln	Gln	Leu	Ala	Gln	Pro	Thr	Gln	Gly	340	345	350	
Thr	Thr	Pro	Ser	Ser	Lys	Leu	Gly	Gly	Leu	Trp	Lys	Thr	Val	Ser	Pro	355	360	365	
His	Arg	Ser	Pro	Ile	Ser	Asn	Met	Val	Ser	Met	Ala	Asn	Asn	His	Met	370	375	380	
Ser	Met	Thr	Asn	Ser	Gly	Val	Ser	Met	Thr	Asn	Thr	Leu	Ser	Ser	Met	385	390	395	400
Leu	Lys	Gly	Phe	Ala	Pro	Ala	Ala	Ala	Ala	Gln	Ala	Val	Gln	Thr	Ala	405	410	415	
Ala	Gln	Asn	Gly	Val	Arg	Ala	Met	Ser	Ser	Leu	Gly	Ser	Ser	Leu	Gly	420	425	430	
Ser	Ser	Gly	Leu	Gly	Gly	Gly	Val	Ala	Ala	Asn	Leu	Gly	Arg	Ala	Ala	435	440	445	
Ser	Val	Gly	Ser	Leu	Ser	Val	Pro	Gln	Ala	Trp	Ala	Ala	Ala	Asn	Gln	450	455	460	
Ala	Val	Thr	Pro	Ala	Ala	Arg	Ala	Leu	Pro	Leu	Thr	Ser	Leu	Thr	Ser	465	470	475	480
Ala	Ala	Glu	Arg	Gly	Pro	Gly	Gln	Met	Leu	Gly	Gly	Leu	Pro	Val	Gly	485	490	495	
Gln	Met	Gly	Ala	Arg	Ala	Gly	Gly	Gly	Leu	Ser	Gly	Val	Leu	Arg	Val	500	505	510	
Pro	Pro	Arg	Pro	Tyr	Val	Met	Pro	His	Ser	Pro	Ala	Ala	Gly	Asp	Ile	515	520	525	
Ala	Pro	Pro	Ala	Leu	Ser	Gln	Asp	Arg	Phe	Ala	Asp	Phe	Pro	Ala	Leu	530	535	540	

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Pro	Leu	Asp	Pro	Ser	Ala	Met	Val	Ala	Gln	Val	Gly	Pro	Gln	Val	Val	545	550	555	560
Asn	Ile	Asn	Thr	Lys	Leu	Gly	Tyr	Asn	Asn	Ala	Val	Gly	Ala	Gly	Thr	565	570	575	
Gly	Ile	Val	Ile	Asp	Pro	Asn	Gly	Val	Val	Leu	Thr	Asn	Asn	His	Val	580	585	590	
Ile	Ala	Gly	Ala	Thr	Asp	Ile	Asn	Ala	Phe	Ser	Val	Gly	Ser	Gly	Gln	595	600	605	
Thr	Tyr	Gly	Val	Asp	Val	Val	Gly	Tyr	Asp	Arg	Thr	Gln	Asp	Val	Ala	610	615	620	
Val	Leu	Gln	Leu	Arg	Gly	Ala	Gly	Gly	Leu	Pro	Ser	Ala	Ala	Ile	Gly	625	630	635	640
Gly	Gly	Val	Ala	Val	Gly	Glu	Pro	Val	Val	Ala	Met	Gly	Asn	Ser	Gly	645	650	655	
Gly	Gln	Gly	Gly	Thr	Pro	Arg	Ala	Val	Pro	Gly	Arg	Val	Val	Ala	Leu	660	665	670	
Gly	Gln	Thr	Val	Gln	Ala	Ser	Asp	Ser	Leu	Thr	Gly	Ala	Glu	Glu	Thr	675	680	685	
Leu	Asn	Gly	Leu	Ile	Gln	Phe	Asp	Ala	Ala	Ile	Gln	Pro	Gly	Asp	Ala	690	695	700	
Gly	Gly	Pro	Val	Val	Asn	Gly	Leu	Gly	Gln	Val	Val	Gly	Met	Asn	Thr	705	710	715	720
Ala	Ala	Ser	Ser	Gly	Phe	Ser	Arg	Pro	Gly	Leu	Pro	Val	Glu	Tyr	Leu	725	730	735	
Gln	Val	Pro	Ser	Pro	Ser	Met	Gly	Arg	Asp	Ile	Lys	Val	Gln	Phe	Gln	740	745	750	
Ser	Gly	Gly	Asn	Asn	Ser	Pro	Ala	Val	Tyr	Leu	Leu	Asp	Gly	Leu	Arg	755	760	765	
Ala	Gln	Asp	Asp	Tyr	Asn	Gly	Trp	Asp	Ile	Asn	Thr	Pro	Ala	Phe	Glu	770	775	780	
Trp	Tyr	Tyr	Gln	Ser	Gly	Leu	Ser	Ile	Val	Met	Pro	Val	Gly	Gly	Gln	785	790	795	800
Ser	Ser	Phe	Tyr	Ser	Asp	Trp	Tyr	Ser	Pro	Ala	Cys	Gly	Lys	Ala	Gly	805	810	815	
Cys	Gln	Thr	Tyr	Lys	Trp	Glu	Thr	Phe	Leu	Thr	Ser	Glu	Leu	Pro	Gln	820	825	830	
Trp	Leu	Ser	Ala	Asn	Arg	Ala	Val	Lys	Pro	Thr	Gly	Ser	Ala	Ala	Ile	835	840	845	
Gly	Leu	Ser	Met	Ala	Gly	Ser	Ser	Ala	Met	Ile	Leu	Ala	Ala	Tyr	His	850	855	860	
Pro	Gln	Gln	Phe	Ile	Tyr	Ala	Gly	Ser	Leu	Ser	Ala	Leu	Leu	Asp	Pro	865	870	875	880
Ser	Gln	Gly	Met	Gly	Pro	Ser	Leu	Ile	Gly	Leu	Ala	Met	Gly	Asp	Ala	885	890	895	
Gly	Gly	Tyr	Lys	Ala	Ala	Asp	Met	Trp	Gly	Pro	Ser	Ser	Asp	Pro	Ala	900	905	910	
Trp	Glu	Arg	Asn	Asp	Pro	Thr	Gln	Gln	Ile	Pro	Lys	Leu	Val	Ala	Asn	915	920	925	
Asn	Thr	Arg	Leu	Trp	Val	Tyr	Cys	Gly	Asn	Gly	Thr	Pro	Asn	Glu	Leu	930	935	940	
Gly	Gly	Ala	Asn	Ile	Pro	Ala	Glu	Phe	Leu	Glu	Asn	Phe	Val	Arg	Ser				

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945          950          955          960
Ser Asn Leu Lys Phe Gln Asp Ala Tyr Asn Ala Ala Gly Gly His Asn
          965          970          975

Ala Val Phe Asn Phe Pro Pro Asn Gly Thr His Ser Trp Glu Tyr Trp
          980          985          990

Gly Ala Gln Leu Asn Ala Met Lys Gly Asp Leu Gln Ser Ser Leu Gly
          995          1000          1005

Ala Gly
  1010

<210> SEQ ID NO 29
<211> LENGTH: 1148
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: M114

<400> SEQUENCE: 29

Met Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly
1          5          10          15

Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg
          20          25          30

Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu
          35          40          45

Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg
          50          55          60

Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp
          65          70          75          80

Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met
          85          90          95

Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr
          100          105          110

Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala
          115          120          125

Glu Gly Pro Pro Ala Glu Phe Met Val Asp Phe Gly Ala Leu Pro Pro
          130          135          140

Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly Pro Gly Ser Ala Ser Leu
          145          150          155          160

Val Ala Ala Ala Gln Met Trp Asp Ser Val Ala Ser Asp Leu Phe Ser
          165          170          175

Ala Ala Ser Ala Phe Gln Ser Val Val Trp Gly Leu Thr Val Gly Ser
          180          185          190

Trp Ile Gly Ser Ser Ala Gly Leu Met Val Ala Ala Ala Ser Pro Tyr
          195          200          205

Val Ala Trp Met Ser Val Thr Ala Gly Gln Ala Glu Leu Thr Ala Ala
          210          215          220

Gln Val Arg Val Ala Ala Ala Ala Tyr Glu Thr Ala Tyr Gly Leu Thr
          225          230          235          240

Val Pro Pro Pro Val Ile Ala Glu Asn Arg Ala Glu Leu Met Ile Leu
          245          250          255

Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr Pro Ala Ile Ala Val Asn
          260          265          270

Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln Asp Ala Ala Ala Met Phe

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275					280					285					
Gly	Tyr	Ala	Ala	Ala	Thr	Ala	Thr	Ala	Thr	Ala	Thr	Leu	Leu	Pro	Phe
	290					295					300				
Glu	Glu	Ala	Pro	Glu	Met	Thr	Ser	Ala	Gly	Gly	Leu	Leu	Glu	Gln	Ala
305					310					315					320
Ala	Ala	Val	Glu	Glu	Ala	Ser	Asp	Thr	Ala	Ala	Ala	Asn	Gln	Leu	Met
				325					330					335	
Asn	Asn	Val	Pro	Gln	Ala	Leu	Gln	Gln	Leu	Ala	Gln	Pro	Thr	Gln	Gly
			340					345					350		
Thr	Thr	Pro	Ser	Ser	Lys	Leu	Gly	Gly	Leu	Trp	Lys	Thr	Val	Ser	Pro
		355					360					365			
His	Arg	Ser	Pro	Ile	Ser	Asn	Met	Val	Ser	Met	Ala	Asn	Asn	His	Met
	370					375					380				
Ser	Met	Thr	Asn	Ser	Gly	Val	Ser	Met	Thr	Asn	Thr	Leu	Ser	Ser	Met
385					390					395					400
Leu	Lys	Gly	Phe	Ala	Pro	Ala	Ala	Ala	Ala	Gln	Ala	Val	Gln	Thr	Ala
				405					410					415	
Ala	Gln	Asn	Gly	Val	Arg	Ala	Met	Ser	Ser	Leu	Gly	Ser	Ser	Leu	Gly
			420					425					430		
Ser	Ser	Gly	Leu	Gly	Gly	Gly	Val	Ala	Ala	Asn	Leu	Gly	Arg	Ala	Ala
		435					440					445			
Ser	Val	Gly	Ser	Leu	Ser	Val	Pro	Gln	Ala	Trp	Ala	Ala	Ala	Asn	Gln
	450					455					460				
Ala	Val	Thr	Pro	Ala	Ala	Arg	Ala	Leu	Pro	Leu	Thr	Ser	Leu	Thr	Ser
465					470					475					480
Ala	Ala	Glu	Arg	Gly	Pro	Gly	Gln	Met	Leu	Gly	Gly	Leu	Pro	Val	Gly
				485					490					495	
Gln	Met	Gly	Ala	Arg	Ala	Gly	Gly	Gly	Leu	Ser	Gly	Val	Leu	Arg	Val
			500					505					510		
Pro	Pro	Arg	Pro	Tyr	Val	Met	Pro	His	Ser	Pro	Ala	Ala	Gly	Asp	Ile
		515					520					525			
Ala	Pro	Pro	Ala	Leu	Ser	Gln	Asp	Arg	Phe	Ala	Asp	Phe	Pro	Ala	Leu
	530					535					540				
Pro	Leu	Asp	Pro	Ser	Ala	Met	Val	Ala	Gln	Val	Gly	Pro	Gln	Val	Val
545					550					555					560
Asn	Ile	Asn	Thr	Lys	Leu	Gly	Tyr	Asn	Asn	Ala	Val	Gly	Ala	Gly	Thr
				565					570					575	
Gly	Ile	Val	Ile	Asp	Pro	Asn	Gly	Val	Val	Leu	Thr	Asn	Asn	His	Val
			580					585					590		
Ile	Ala	Gly	Ala	Thr	Asp	Ile	Asn	Ala	Phe	Ser	Val	Gly	Ser	Gly	Gln
		595					600					605			
Thr	Tyr	Gly	Val	Asp	Val	Val	Gly	Tyr	Asp	Arg	Thr	Gln	Asp	Val	Ala
	610					615					620				
Val	Leu	Gln	Leu	Arg	Gly	Ala	Gly	Gly	Leu	Pro	Ser	Ala	Ala	Ile	Gly
625					630					635					640
Gly	Gly	Val	Ala	Val	Gly	Glu	Pro	Val	Val	Ala	Met	Gly	Asn	Ser	Gly
				645					650					655	
Gly	Gln	Gly	Gly	Thr	Pro	Arg	Ala	Val	Pro	Gly	Arg	Val	Val	Ala	Leu
			660					665					670		
Gly	Gln	Thr	Val	Gln	Ala	Ser	Asp	Ser	Leu	Thr	Gly	Ala	Glu	Glu	Thr
		675					680					685			

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Leu	Asn	Gly	Leu	Ile	Gln	Phe	Asp	Ala	Ala	Ile	Gln	Pro	Gly	Asp	Ala		
690						695					700						
Gly	Gly	Pro	Val	Val	Asn	Gly	Leu	Gly	Gln	Val	Val	Gly	Met	Asn	Thr		
705					710				715						720		
Ala	Ala	Ser	Ser	Thr	Met	Asp	Phe	Gly	Leu	Leu	Pro	Pro	Glu	Val	Asn		
				725					730					735			
Ser	Ser	Arg	Met	Tyr	Ser	Gly	Pro	Gly	Pro	Glu	Ser	Met	Leu	Ala	Ala		
			740					745					750				
Ala	Ala	Ala	Trp	Asp	Gly	Val	Ala	Ala	Glu	Leu	Thr	Ser	Ala	Ala	Val		
		755				760					765						
Ser	Tyr	Gly	Ser	Val	Val	Ser	Thr	Leu	Ile	Val	Glu	Pro	Trp	Met	Gly		
770						775					780						
Pro	Ala	Ala	Ala	Ala	Met	Ala	Ala	Ala	Ala	Thr	Pro	Tyr	Val	Gly	Trp		
785					790					795					800		
Leu	Ala	Ala	Thr	Ala	Ala	Leu	Ala	Lys	Glu	Thr	Ala	Thr	Gln	Ala	Arg		
			805						810					815			
Ala	Ala	Ala	Glu	Ala	Phe	Gly	Thr	Ala	Phe	Ala	Met	Thr	Val	Pro	Pro		
		820					825					830					
Ser	Leu	Val	Ala	Ala	Asn	Arg	Ser	Arg	Leu	Met	Ser	Leu	Val	Ala	Ala		
		835				840						845					
Asn	Ile	Leu	Gly	Gln	Asn	Ser	Ala	Ala	Ile	Ala	Ala	Thr	Gln	Ala	Glu		
850					855					860							
Tyr	Ala	Glu	Met	Trp	Ala	Gln	Asp	Ala	Ala	Val	Met	Tyr	Ser	Tyr	Glu		
865				870					875					880			
Gly	Ala	Ser	Ala	Ala	Ala	Ser	Ala	Leu	Pro	Phe	Thr	Pro	Pro	Val			
			885					890				895					
Gln	Gly	Thr	Gly	Pro	Ala	Gly	Pro	Ala	Ala	Ala	Ala	Ala	Ala	Thr	Gln		
		900					905					910					
Ala	Ala	Gly	Ala	Gly	Ala	Val	Ala	Asp	Ala	Gln	Ala	Thr	Leu	Ala	Gln		
		915				920					925						
Leu	Pro	Pro	Gly	Ile	Leu	Ser	Asp	Ile	Leu	Ser	Ala	Leu	Ala	Ala	Asn		
930					935					940							
Ala	Asp	Pro	Leu	Thr	Ser	Gly	Leu	Leu	Gly	Ile	Ala	Ser	Thr	Leu	Asn		
945				950					955					960			
Pro	Gln	Val	Gly	Ser	Ala	Gln	Pro	Ile	Val	Ile	Pro	Thr	Pro	Ile	Gly		
			965					970					975				
Glu	Leu	Asp	Val	Ile	Ala	Leu	Tyr	Ile	Ala	Ser	Ile	Ala	Thr	Gly	Ser		
		980					985					990					
Ile	Ala	Leu	Ala	Ile	Thr	Asn	Thr	Ala	Arg	Pro	Trp	His	Ile	Gly	Leu		
		995				1000						1005					
Tyr	Gly	Asn	Ala	Gly	Gly	Leu	Gly	Pro	Thr	Gln	Gly	His	Pro	Leu			
1010						1015					1020						
Ser	Ser	Ala	Thr	Asp	Glu	Pro	Glu	Pro	His	Trp	Gly	Pro	Phe	Gly			
1025						1030					1035						
Gly	Ala	Ala	Pro	Val	Ser	Ala	Gly	Val	Gly	His	Ala	Ala	Leu	Val			
1040						1045					1050						
Gly	Ala	Leu	Ser	Val	Pro	His	Ser	Trp	Thr	Thr	Ala	Ala	Pro	Glu			
1055						1060					1065						
Ile	Gln	Leu	Ala	Val	Gln	Ala	Thr	Pro	Thr	Phe	Ser	Ser	Ser	Ala			
1070						1075					1080						

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Gly	Ala	Asp	Pro	Thr	Ala	Leu	Asn	Gly	Met	Pro	Ala	Gly	Leu	Leu
1085						1090					1095			
Ser	Gly	Met	Ala	Leu	Ala	Ser	Leu	Ala	Ala	Arg	Gly	Thr	Thr	Gly
1100						1105					1110			
Gly	Gly	Gly	Thr	Arg	Ser	Gly	Thr	Ser	Thr	Asp	Gly	Gln	Glu	Asp
1115						1120					1125			
Gly	Arg	Lys	Pro	Pro	Val	Val	Val	Ile	Arg	Glu	Gln	Pro	Pro	Pro
1130						1135					1140			
Gly	Asn	Pro	Pro	Arg										
1145														

<210> SEQ ID NO 30
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 30

Trp	Val	Leu	Ala	Ala	Gly	Val	Gln	Ala
1				5				

<210> SEQ ID NO 31
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 31

Val	Leu	Ala	Ala	Gly	Val	Gln	Ala	Met
1				5				

<210> SEQ ID NO 32
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 32

Val	Ile	Arg	Asp	Gly	Val	Thr	Arg	Arg
1				5				

<210> SEQ ID NO 33
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 33

Leu	Ala	Pro	His	Thr	Pro	Leu	Ala	Arg
1				5				

<210> SEQ ID NO 34
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 34

Ile	Arg	Leu	Phe	Asp	Ala	Gly	Ile	Arg
1				5				

<210> SEQ ID NO 35
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis

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<400> SEQUENCE: 35

Ile Arg His Arg Glu Ala Ile Asp Arg
1 5

<210> SEQ ID NO 36

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 36

Leu Leu Ala Thr Gly Val Arg Glu Val
1 5

<210> SEQ ID NO 37

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 37

Phe Arg Arg Arg Val Ala Val Ala Val
1 5

<210> SEQ ID NO 38

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 38

Phe Ala Ile Asp Phe Pro Leu Thr Tyr
1 5

<210> SEQ ID NO 39

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 39

Phe Pro Leu Thr Tyr Arg Leu Gly Arg
1 5

<210> SEQ ID NO 40

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 40

Tyr Arg Leu Gly Arg Arg His Asn Thr
1 5

<210> SEQ ID NO 41

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 41

Val Arg Ser Phe Leu Leu Gln Leu Gly
1 5

<210> SEQ ID NO 42

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

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<400> SEQUENCE: 42

Leu Leu Gln Leu Gly Gly Ile Arg Ala
1 5

<210> SEQ ID NO 43

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 43

Leu Val Thr Ala Val Arg Ala Asp Gly
1 5

<210> SEQ ID NO 44

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 44

Val Arg Ala Asp Gly Val Val Ile Thr
1 5

<210> SEQ ID NO 45

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 45

Val Val Ile Thr Glu Pro Leu Ala Gly
1 5

<210> SEQ ID NO 46

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 46

Ile Val Glu His Ala Ile Ser Val Arg
1 5

<210> SEQ ID NO 47

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 47

Ile Asp Phe Met Thr Val Arg Glu Arg
1 5

<210> SEQ ID NO 48

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 48

Phe Arg Leu Asp Glu Cys Pro Arg Gly
1 5

<210> SEQ ID NO 49

<211> LENGTH: 9

<212> TYPE: PRT

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<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 49

Leu Tyr Ser Gly Ala Val Val Met Leu
1 5

<210> SEQ ID NO 50

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 50

Tyr Ser Gly Ala Val Val Met Leu Ser
1 5

<210> SEQ ID NO 51

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 51

Val Val Met Leu Ser Ala Asp Gly Gly
1 5

<210> SEQ ID NO 52

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 52

Trp Leu Arg Ala Gly Ala Gly Ile Ile
1 5

<210> SEQ ID NO 53

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 53

Glu Leu Ser Val Ala Thr Gly Ala Val
1 5

<210> SEQ ID NO 54

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 54

Ser Val Ala Thr Gly Ala Val Ser Thr
1 5

<210> SEQ ID NO 55

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 55

Ile Pro Met Pro Ala Gly Val Asn Pro
1 5

<210> SEQ ID NO 56

<211> LENGTH: 9

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<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 56

Pro Met Pro Ala Gly Val Asn Pro Ala
1 5

<210> SEQ ID NO 57
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 57

Pro Ala Gly Val Asn Pro Ala Asp Leu
1 5

<210> SEQ ID NO 58
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 58

Gly Val Asn Pro Ala Asp Leu Ala Ala
1 5

<210> SEQ ID NO 59
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 59

Asn Pro Ala Asp Leu Ala Ala Glu Leu
1 5

<210> SEQ ID NO 60
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 60

Asp Leu Ala Ala Glu Leu Ala Ala Val
1 5

<210> SEQ ID NO 61
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 61

Leu Ala Ala Val Val Thr Glu Ser Val
1 5

<210> SEQ ID NO 62
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 62

Val Thr Glu Ser Val Asp Glu Asp Tyr
1 5

<210> SEQ ID NO 63

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<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 63

Thr Glu Ser Val Asp Glu Asp Tyr Leu
1 5

<210> SEQ ID NO 64
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 64

Ser Val Asp Glu Asp Tyr Leu Leu Tyr
1 5

<210> SEQ ID NO 65
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 65

Tyr Glu Cys Asp Gly Gln Trp Val Leu
1 5

<210> SEQ ID NO 66
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 66

Gly Gln Trp Val Leu Ala Ala Gly Val
1 5

<210> SEQ ID NO 67
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 67

Val Leu Ala Ala Gly Val Gln Ala Met
1 5

<210> SEQ ID NO 68
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 68

Glu Leu Asp Ser Asp Glu Leu Arg Val
1 5

<210> SEQ ID NO 69
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 69

Asp Ser Asp Glu Leu Arg Val Ile Arg
1 5

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<210> SEQ ID NO 70
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 70

Gln Gln Trp Ser Gly Arg Pro Gly Ala
1 5

<210> SEQ ID NO 71
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 71

Gly Arg Pro Gly Ala Ala Leu Gly Glu
1 5

<210> SEQ ID NO 72
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 72

Arg Pro Gly Ala Ala Leu Gly Glu Ala
1 5

<210> SEQ ID NO 73
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 73

Ala Leu Gly Glu Ala Val Asp Arg Leu
1 5

<210> SEQ ID NO 74
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 74

Gly Glu Ala Val Asp Arg Leu Leu Leu
1 5

<210> SEQ ID NO 75
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 75

Ala Phe Gly Trp Val Ala Phe Glu Phe
1 5

<210> SEQ ID NO 76
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 76

Arg Leu Ala Pro His Thr Pro Leu Ala
1 5

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<210> SEQ ID NO 77
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 77

Leu Ala Pro His Thr Pro Leu Ala Arg
1 5

<210> SEQ ID NO 78
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 78

Ala Pro His Thr Pro Leu Ala Arg Val
1 5

<210> SEQ ID NO 79
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 79

Pro His Thr Pro Leu Ala Arg Val Phe
1 5

<210> SEQ ID NO 80
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 80

Pro Leu Ala Arg Val Phe Ser Pro Arg
1 5

<210> SEQ ID NO 81
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 81

Arg Val Phe Ser Pro Arg Thr Arg Ile
1 5

<210> SEQ ID NO 82
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 82

Val Phe Ser Pro Arg Thr Arg Ile Met
1 5

<210> SEQ ID NO 83
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 83

Val Ser Glu Lys Glu Ile Arg Leu Phe
1 5

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<210> SEQ ID NO 84
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 84

Ala Gly Ile Arg His Arg Glu Ala Ile
1 5

<210> SEQ ID NO 85
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 85

Leu Leu Ala Thr Gly Val Arg Glu Val
1 5

<210> SEQ ID NO 86
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 86

Arg Glu Val Pro Gln Ser Arg Ser Val
1 5

<210> SEQ ID NO 87
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 87

Val Pro Gln Ser Arg Ser Val Asp Val
1 5

<210> SEQ ID NO 88
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 88

Val Ser Asp Asp Pro Ser Gly Phe Arg
1 5

<210> SEQ ID NO 89
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 89

Asp Pro Ser Gly Phe Arg Arg Arg Val
1 5

<210> SEQ ID NO 90
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 90

Ser Gly Phe Arg Arg Arg Val Ala Val

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1 5

<210> SEQ ID NO 91
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 91

Gly Phe Arg Arg Arg Val Ala Val Ala
1 5

<210> SEQ ID NO 92
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 92

Phe Arg Arg Arg Val Ala Val Ala Val
1 5

<210> SEQ ID NO 93
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 93

Arg Val Ala Val Ala Val Asp Glu Ile
1 5

<210> SEQ ID NO 94
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 94

Ile Ala Ala Gly Arg Tyr His Lys Val
1 5

<210> SEQ ID NO 95
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 95

Ala Gly Arg Tyr His Lys Val Ile Leu
1 5

<210> SEQ ID NO 96
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 96

Arg Tyr His Lys Val Ile Leu Ser Arg
1 5

<210> SEQ ID NO 97
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 97

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Val Ile Leu Ser Arg Cys Val Glu Val
1 5

<210> SEQ ID NO 98
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 98

Val Pro Phe Ala Ile Asp Phe Pro Leu
1 5

<210> SEQ ID NO 99
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 99

Phe Ala Ile Asp Phe Pro Leu Thr Tyr
1 5

<210> SEQ ID NO 100
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 100

Ile Asp Phe Pro Leu Thr Tyr Arg Leu
1 5

<210> SEQ ID NO 101
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 101

Leu Gly Arg Arg His Asn Thr Pro Val
1 5

<210> SEQ ID NO 102
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 102

Arg Arg His Asn Thr Pro Val Arg Ser
1 5

<210> SEQ ID NO 103
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 103

Asn Thr Pro Val Arg Ser Phe Leu Leu
1 5

<210> SEQ ID NO 104
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 104

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Pro Val Arg Ser Phe Leu Leu Gln Leu
1 5

<210> SEQ ID NO 105
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 105

Arg Ala Leu Gly Tyr Ser Pro Glu Leu
1 5

<210> SEQ ID NO 106
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 106

Ala Leu Gly Tyr Ser Pro Glu Leu Val
1 5

<210> SEQ ID NO 107
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 107

Tyr Ser Pro Glu Leu Val Thr Ala Val
1 5

<210> SEQ ID NO 108
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 108

Ser Pro Glu Leu Val Thr Ala Val Arg
1 5

<210> SEQ ID NO 109
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 109

Ala Val Arg Ala Asp Gly Val Val Ile
1 5

<210> SEQ ID NO 110
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 110

Ile Thr Glu Pro Leu Ala Gly Thr Arg
1 5

<210> SEQ ID NO 111
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

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<400> SEQUENCE: 111

Glu Pro Leu Ala Gly Thr Arg Ala Leu
1 5

<210> SEQ ID NO 112

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 112

Gly Thr Arg Ala Leu Gly Arg Gly Pro
1 5

<210> SEQ ID NO 113

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 113

Arg Ala Leu Gly Arg Gly Pro Ala Ile
1 5

<210> SEQ ID NO 114

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 114

Leu Glu Ser Asn Ser Lys Glu Ile Val
1 5

<210> SEQ ID NO 115

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 115

Glu Ile Val Glu His Ala Ile Ser Val
1 5

<210> SEQ ID NO 116

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 116

Ile Val Glu His Ala Ile Ser Val Arg
1 5

<210> SEQ ID NO 117

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 117

His Ala Ile Ser Val Arg Ser Ser Leu
1 5

<210> SEQ ID NO 118

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

-continued

<400> SEQUENCE: 118

Ser Val Arg Ser Ser Leu Glu Glu Ile
1 5

<210> SEQ ID NO 119

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 119

Ser Leu Glu Glu Ile Thr Asp Ile Ala
1 5

<210> SEQ ID NO 120

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 120

Asp Ile Ala Glu Pro Gly Ser Ala Ala
1 5

<210> SEQ ID NO 121

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 121

Ala Glu Pro Gly Ser Ala Ala Val Ile
1 5

<210> SEQ ID NO 122

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 122

Arg Glu Arg Gly Ser Val Gln His Leu
1 5

<210> SEQ ID NO 123

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 123

Ser Val Gln His Leu Gly Ser Thr Ile
1 5

<210> SEQ ID NO 124

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 124

Arg Leu Asp Pro Ser Ser Asp Arg Met
1 5

<210> SEQ ID NO 125

<211> LENGTH: 9

<212> TYPE: PRT

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<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 125

Asp Pro Ser Ser Asp Arg Met Ala Ala
1 5

<210> SEQ ID NO 126

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 126

Ser Ser Asp Arg Met Ala Ala Leu Glu
1 5

<210> SEQ ID NO 127

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 127

Asp Arg Met Ala Ala Leu Glu Ala Leu
1 5

<210> SEQ ID NO 128

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 128

Arg Met Ala Ala Leu Glu Ala Leu Phe
1 5

<210> SEQ ID NO 129

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 129

Ala Ala Leu Glu Ala Leu Phe Pro Ala
1 5

<210> SEQ ID NO 130

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 130

Ala Leu Glu Ala Leu Phe Pro Ala Val
1 5

<210> SEQ ID NO 131

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 131

Leu Glu Ala Leu Phe Pro Ala Val Thr
1 5

<210> SEQ ID NO 132

<211> LENGTH: 9

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<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 132

Phe Pro Ala Val Thr Ala Ser Gly Ile
1 5

<210> SEQ ID NO 133
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 133

Ala Val Thr Ala Ser Gly Ile Pro Lys
1 5

<210> SEQ ID NO 134
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 134

Val Thr Ala Ser Gly Ile Pro Lys Ala
1 5

<210> SEQ ID NO 135
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 135

Gly Ile Pro Lys Ala Ala Gly Val Glu
1 5

<210> SEQ ID NO 136
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 136

Ile Pro Lys Ala Ala Gly Val Glu Ala
1 5

<210> SEQ ID NO 137
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 137

Lys Ala Ala Gly Val Glu Ala Ile Phe
1 5

<210> SEQ ID NO 138
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 138

Ala Gly Val Glu Ala Ile Phe Arg Leu
1 5

<210> SEQ ID NO 139

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<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 139

Arg Leu Asp Glu Cys Pro Arg Gly Leu
1 5

<210> SEQ ID NO 140
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 140

Cys Pro Arg Gly Leu Tyr Ser Gly Ala
1 5

<210> SEQ ID NO 141
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 141

Gly Leu Tyr Ser Gly Ala Val Val Met
1 5

<210> SEQ ID NO 142
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 142

Leu Tyr Ser Gly Ala Val Val Met Leu
1 5

<210> SEQ ID NO 143
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 143

Val Met Leu Ser Ala Asp Gly Gly Leu
1 5

<210> SEQ ID NO 144
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 144

Gly Leu Asp Ala Ala Leu Thr Leu Arg
1 5

<210> SEQ ID NO 145
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 145

Leu Asp Ala Ala Leu Thr Leu Arg Ala
1 5

-continued

<210> SEQ ID NO 146
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 146

Ala Ala Leu Thr Leu Arg Ala Ala Tyr
1 5

<210> SEQ ID NO 147
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 147

Leu Thr Leu Arg Ala Ala Tyr Gln Val
1 5

<210> SEQ ID NO 148
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 148

Ala Tyr Gln Val Gly Gly Arg Thr Trp
1 5

<210> SEQ ID NO 149
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 149

Thr Trp Leu Arg Ala Gly Ala Gly Ile
1 5

<210> SEQ ID NO 150
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 150

Glu Glu Ser Glu Pro Glu Arg Glu Phe
1 5

<210> SEQ ID NO 151
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 151

Arg Glu Phe Glu Glu Thr Cys Glu Lys
1 5

<210> SEQ ID NO 152
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 152

Lys Leu Ser Thr Leu Thr Pro Tyr Leu
1 5

-continued

<210> SEQ ID NO 153
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 153

Ser Thr Leu Thr Pro Tyr Leu Val Ala
1 5

<210> SEQ ID NO 154
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 154

Thr Leu Thr Pro Tyr Leu Val Ala Arg
1 5

<210> SEQ ID NO 155
<211> LENGTH: 1053
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 155

Met Asn Phe Ser Val Leu Pro Pro Glu Ile Asn Ser Ala Leu Ile Phe
1 5 10 15

Ala Gly Ala Gly Pro Glu Pro Met Ala Ala Ala Thr Ala Trp Asp
20 25 30

Gly Leu Ala Met Glu Leu Ala Ser Ala Ala Ala Ser Phe Gly Ser Val
35 40 45

Thr Ser Gly Leu Val Gly Gly Ala Trp Gln Gly Ala Ser Ser Ser Ala
50 55 60

Met Ala Ala Ala Ala Pro Tyr Ala Ala Trp Leu Ala Ala Ala Ala
65 70 75 80

Val Gln Ala Glu Gln Thr Ala Ala Gln Ala Ala Met Ile Ala Glu
85 90 95

Phe Glu Ala Val Lys Thr Ala Val Val Gln Pro Met Leu Val Ala Ala
100 105 110

Asn Arg Ala Asp Leu Val Ser Leu Val Met Ser Asn Leu Phe Gly Gln
115 120 125

Asn Ala Pro Ala Ile Ala Ala Ile Glu Ala Thr Tyr Glu Gln Met Trp
130 135 140

Ala Ala Asp Val Ser Ala Met Ser Ala Tyr His Ala Gly Ala Ser Ala
145 150 155 160

Ile Ala Ser Ala Leu Ser Pro Phe Ser Lys Pro Leu Gln Asn Leu Ala
165 170 175

Gly Leu Pro Ala Trp Leu Ala Ser Gly Ala Pro Ala Ala Ala Met Thr
180 185 190

Ala Ala Ala Gly Ile Pro Ala Leu Ala Gly Gly Pro Thr Ala Ile Asn
195 200 205

Leu Gly Ile Ala Asn Val Gly Gly Gly Asn Val Gly Asn Ala Asn Asn
210 215 220

Gly Leu Ala Asn Ile Gly Asn Ala Asn Leu Gly Asn Tyr Asn Phe Gly
225 230 235 240

Ser Gly Asn Phe Gly Asn Ser Asn Ile Gly Ser Ala Ser Leu Gly Asn

-continued

245							250					255				
Asn	Asn	Ile	Gly	Phe	Gly	Asn	Leu	Gly	Ser	Asn	Asn	Val	Gly	Val	Gly	
			260					265					270			
Asn	Leu	Gly	Asn	Leu	Asn	Thr	Gly	Phe	Ala	Asn	Thr	Gly	Leu	Gly	Asn	
		275					280					285				
Phe	Gly	Phe	Gly	Asn	Thr	Gly	Asn	Asn	Asn	Ile	Gly	Ile	Gly	Leu	Thr	
	290					295					300					
Gly	Asn	Asn	Gln	Ile	Gly	Ile	Gly	Gly	Leu	Asn	Ser	Gly	Thr	Gly	Asn	
305					310					315					320	
Phe	Gly	Leu	Phe	Asn	Ser	Gly	Ser	Gly	Asn	Val	Gly	Phe	Phe	Asn	Ser	
				325					330					335		
Gly	Asn	Gly	Asn	Phe	Gly	Ile	Gly	Asn	Ser	Gly	Asn	Phe	Asn	Thr	Gly	
			340				345						350			
Gly	Trp	Asn	Ser	Gly	His	Gly	Asn	Thr	Gly	Phe	Phe	Asn	Ala	Gly	Ser	
	355					360						365				
Phe	Asn	Thr	Gly	Met	Leu	Asp	Val	Gly	Asn	Ala	Asn	Thr	Gly	Ser	Leu	
	370					375					380					
Asn	Thr	Gly	Ser	Tyr	Asn	Met	Gly	Asp	Phe	Asn	Pro	Gly	Ser	Ser	Asn	
385					390					395					400	
Thr	Gly	Thr	Phe	Asn	Thr	Gly	Asn	Ala	Asn	Thr	Gly	Phe	Leu	Asn	Ala	
				405					410					415		
Gly	Asn	Ile	Asn	Thr	Gly	Val	Phe	Asn	Ile	Gly	His	Met	Asn	Asn	Gly	
		420						425					430			
Leu	Phe	Asn	Thr	Gly	Asp	Met	Asn	Asn	Gly	Val	Phe	Tyr	Arg	Gly	Val	
	435						440					445				
Gly	Gln	Gly	Ser	Leu	Gln	Phe	Ser	Ile	Thr	Thr	Pro	Asp	Leu	Thr	Leu	
	450					455					460					
Pro	Pro	Leu	Gln	Ile	Pro	Gly	Ile	Ser	Val	Pro	Ala	Phe	Ser	Leu	Pro	
465					470					475					480	
Ala	Ile	Thr	Leu	Pro	Ser	Leu	Asn	Ile	Pro	Ala	Ala	Thr	Thr	Pro	Ala	
				485					490					495		
Asn	Ile	Thr	Val	Gly	Ala	Phe	Ser	Leu	Pro	Gly	Leu	Thr	Leu	Pro	Ser	
			500					505					510			
Leu	Asn	Ile	Pro	Ala	Ala	Thr	Thr	Pro	Ala	Asn	Ile	Thr	Val	Gly	Ala	
	515						520					525				
Phe	Ser	Leu	Pro	Gly	Leu	Thr	Leu	Pro	Ser	Leu	Asn	Ile	Pro	Ala	Ala	
	530					535					540					
Thr	Thr	Pro	Ala	Asn	Ile	Thr	Val	Gly	Ala	Phe	Ser	Leu	Pro	Gly	Leu	
545					550					555					560	
Thr	Leu	Pro	Ser	Leu	Asn	Ile	Pro	Ala	Ala	Thr	Thr	Pro	Ala	Asn	Ile	
				565					570					575		
Thr	Val	Gly	Ala	Phe	Ser	Leu	Pro	Gly	Leu	Thr	Leu	Pro	Ser	Leu	Asn	
		580						585					590			
Ile	Pro	Ala	Ala	Thr	Thr	Pro	Ala	Asn	Ile	Thr	Val	Gly	Ala	Phe	Ser	
		595					600					605				
Leu	Pro	Gly	Leu	Thr	Leu	Pro	Ser	Leu	Asn	Ile	Pro	Ala	Ala	Thr	Thr	
	610					615					620					
Pro	Ala	Asn	Ile	Thr	Val	Ser	Gly	Phe	Gln	Leu	Pro	Pro	Leu	Ser	Ile	
625					630					635					640	
Pro	Ser	Val	Ala	Ile	Pro	Pro	Val	Thr	Val	Pro	Pro	Ile	Thr	Val	Gly	
				645					650					655		

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Ala Phe Asn Leu Pro Pro Leu Gln Ile Pro Glu Val Thr Ile Pro Gln		
660	665	670
Leu Thr Ile Pro Ala Gly Ile Thr Ile Gly Gly Phe Ser Leu Pro Ala		
675	680	685
Ile His Thr Gln Pro Ile Thr Val Gly Gln Ile Gly Val Gly Gln Phe		
690	695	700
Gly Leu Pro Ser Ile Gly Trp Asp Val Phe Leu Ser Thr Pro Arg Ile		
705	710	715
Thr Val Pro Ala Phe Gly Ile Pro Phe Thr Leu Gln Phe Gln Thr Asn		
725	730	735
Val Pro Ala Leu Gln Pro Pro Gly Gly Gly Leu Ser Thr Phe Thr Asn		
740	745	750
Gly Ala Leu Ile Phe Gly Glu Phe Asp Leu Pro Gln Leu Val Val His		
755	760	765
Pro Tyr Thr Leu Thr Gly Pro Ile Val Ile Gly Ser Phe Phe Leu Pro		
770	775	780
Ala Phe Asn Ile Pro Gly Ile Asp Val Pro Ala Ile Asn Val Asp Gly		
785	790	795
Phe Thr Leu Pro Gln Ile Thr Thr Pro Ala Ile Thr Thr Pro Glu Phe		
805	810	815
Ala Ile Pro Pro Ile Gly Val Gly Gly Phe Thr Leu Pro Gln Ile Thr		
820	825	830
Thr Gln Glu Ile Ile Thr Pro Glu Leu Thr Ile Asn Ser Ile Gly Val		
835	840	845
Gly Gly Phe Thr Leu Pro Gln Ile Thr Thr Pro Pro Ile Thr Thr Pro		
850	855	860
Pro Leu Thr Ile Asp Pro Ile Asn Leu Thr Gly Phe Thr Leu Pro Gln		
865	870	875
Ile Thr Thr Pro Pro Ile Thr Thr Pro Pro Leu Thr Ile Asp Pro Ile		
885	890	895
Asn Leu Thr Gly Phe Thr Leu Pro Gln Ile Thr Thr Pro Pro Ile Thr		
900	905	910
Thr Pro Pro Leu Thr Ile Glu Pro Ile Gly Val Gly Gly Phe Thr Thr		
915	920	925
Pro Pro Leu Thr Val Pro Gly Ile His Leu Pro Ser Thr Thr Ile Gly		
930	935	940
Ala Phe Ala Ile Pro Gly Gly Pro Gly Tyr Phe Asn Ser Ser Thr Ala		
945	950	955
Pro Ser Ser Gly Phe Phe Asn Ser Gly Ala Gly Gly Asn Ser Gly Phe		
965	970	975
Gly Asn Asn Gly Ser Gly Leu Ser Gly Trp Phe Asn Thr Asn Pro Ala		
980	985	990
Gly Leu Leu Gly Gly Ser Gly Tyr Gln Asn Phe Gly Gly Leu Ser Ser		
995	1000	1005
Gly Phe Ser Asn Leu Gly Ser Gly Val Ser Gly Phe Ala Asn Arg		
1010	1015	1020
Gly Ile Leu Pro Phe Ser Val Ala Ser Val Val Ser Gly Phe Ala		
1025	1030	1035
Asn Ile Gly Thr Asn Leu Ala Gly Phe Phe Gln Gly Thr Thr Ser		
1040	1045	1050

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<210> SEQ ID NO 156
<211> LENGTH: 324
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 156

Met Ser Asp Gln Val Pro Lys Pro His Arg His His Ile Trp Arg Ile
1          5          10          15

Thr Arg Arg Thr Leu Ser Lys Ser Trp Asp Asp Ser Ile Phe Ser Glu
20          25          30

Ser Ala Gln Ala Ala Phe Trp Ser Ala Leu Ser Leu Pro Pro Leu Leu
35          40          45

Leu Gly Met Leu Gly Ser Leu Ala Tyr Val Ala Pro Leu Phe Gly Pro
50          55          60

Asp Thr Leu Pro Ala Ile Glu Lys Ser Ala Leu Ser Thr Ala His Ser
65          70          75          80

Phe Phe Ser Pro Ser Val Val Asn Glu Ile Ile Glu Pro Thr Ile Gly
85          90          95

Asp Ile Thr Asn Asn Ala Arg Gly Glu Val Ala Ser Leu Gly Phe Leu
100         105         110

Ile Ser Leu Trp Ala Gly Ser Ser Ala Ile Ser Ala Phe Val Asp Ala
115         120         125

Val Val Glu Ala His Asp Gln Thr Pro Leu Arg His Pro Val Arg Gln
130         135         140

Arg Phe Phe Ala Leu Phe Leu Tyr Val Val Met Leu Val Phe Leu Val
145         150         155         160

Ala Thr Ala Pro Val Met Val Val Gly Pro Arg Lys Val Ser Glu His
165         170         175

Ile Pro Glu Ser Leu Ala Asn Leu Leu Arg Tyr Gly Tyr Tyr Pro Ala
180         185         190

Leu Ile Leu Gly Leu Thr Val Gly Val Ile Leu Leu Tyr Arg Val Ala
195         200         205

Leu Pro Val Pro Leu Pro Thr His Arg Leu Val Leu Gly Ala Val Leu
210         215         220

Ala Ile Ala Val Phe Leu Ile Ala Thr Leu Gly Leu Arg Val Tyr Leu
225         230         235         240

Ala Trp Ile Thr Arg Thr Gly Tyr Thr Tyr Gly Ala Leu Ala Thr Pro
245         250         255

Ile Ala Phe Leu Leu Phe Ala Phe Phe Gly Gly Phe Ala Ile Met Leu
260         265         270

Gly Ala Glu Leu Asn Ala Ala Val Gln Glu Glu Trp Pro Ala Pro Ala
275         280         285

Thr His Ala His Arg Leu Gly Asn Trp Leu Lys Ala Arg Ile Gly Val
290         295         300

Gly Thr Thr Thr Tyr Ser Ser Thr Ala Gln His Ser Ala Val Ala Ala
305         310         315         320

Glu Pro Pro Ser

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1-50. (canceled)

51. A pharmaceutical composition comprising:

(a) a polypeptide which comprises:

- (i) an Rv2386c protein sequence;
- (ii) a variant of an Rv2386c protein sequence; or
- (iii) an immunogenic fragment of an Rv2386c protein sequence; or

(b) a polynucleotide comprising a nucleic acid sequence encoding the polypeptide of (a);

and a pharmaceutically acceptable carrier or excipient.

52. An immunogenic composition comprising:

(a) a polypeptide which comprises:

- (i) an Rv2386c protein sequence;
- (ii) a variant of an Rv2386c protein sequence; or
- (iii) an immunogenic fragment of an Rv2386c protein sequence; or

(b) a polynucleotide comprising a nucleic acid sequence encoding the polypeptide of (a);

and a non-specific immune response enhancer.

53-59. (canceled)

60. A method for the treatment or prevention of tuberculosis comprising the administration of a polypeptide comprising:

- (i) an Rv2386c protein sequence;
- (ii) a variant of an Rv2386c protein sequence; or
- (iii) an immunogenic fragment of an Rv2386c protein sequence;

to a subject in need thereof, wherein said polypeptide induces an immune response.

61. A method for the treatment or prevention of tuberculosis comprising the administration of a polynucleotide comprising a nucleic acid sequence encoding a polypeptide comprising:

- (i) an Rv2386c protein sequence;
- (ii) a variant of an Rv2386c protein sequence; or
- (iii) an immunogenic fragment of an Rv2386c protein sequence;

to a subject in need thereof, wherein said polynucleotide induces an immune response.

62-65. (canceled)

66. The method according to claim 60, wherein the subject has active tuberculosis.

67. The method according to claim 60, wherein the subject has latent tuberculosis.

68. The method according to claim 60, wherein the subject does not have tuberculosis.

69-80. (canceled)

81. The method according to claim 60, wherein the treatment or prevention of tuberculosis refers to the treatment of tuberculosis.

82. The method according to claim 60, wherein the treatment or prevention of tuberculosis refers to the prevention of tuberculosis.

83. The method according to claim 60, wherein the treatment or prevention of tuberculosis refers to the treatment of latent tuberculosis.

84. The method according to claim 60, wherein the treatment or prevention of tuberculosis refers to the prevention of latent tuberculosis.

85. The method according to claim 60, wherein the treatment or prevention of tuberculosis refers to the prevention of reactivation of tuberculosis.

86. The method according to claim 60, wherein the treatment or prevention of tuberculosis refers to the delay of reactivation of tuberculosis.

87-91. (canceled)

92. A fusion protein comprising a polypeptide comprising:

- (i) an Rv2386c protein sequence;
- (ii) a variant of an Rv2386c protein sequence; or
- (iii) an immunogenic fragment of an Rv2386c protein sequence.

93. A polynucleotide comprising a nucleotide sequence encoding a fusion protein comprising a polypeptide comprising:

- (i) an Rv2386c protein sequence;
- (ii) a variant of an Rv2386c protein sequence; or
- (iii) an immunogenic fragment of an Rv2386c protein sequence.

94-103. (canceled)

104. The method according to claim 61, wherein the subject has active tuberculosis.

105. The method according to claim 61, wherein the subject has latent tuberculosis.

106. The method according to claim 61, wherein the subject does not have tuberculosis.

107. The method according to claim 61, wherein the treatment or prevention of tuberculosis refers to the treatment of tuberculosis.

108. The method according to claim 61, wherein the treatment or prevention of tuberculosis refers to the prevention of tuberculosis.

109. The method according to claim 61, wherein the treatment or prevention of tuberculosis refers to the treatment of latent tuberculosis.

110. The method according to claim 61, wherein the treatment or prevention of tuberculosis refers to the prevention of latent tuberculosis.

111. The method according to claim 61, wherein the treatment or prevention of tuberculosis refers to the prevention of reactivation of tuberculosis.

112. The method according to claim 61, wherein the treatment or prevention of tuberculosis refers to the delay of reactivation of tuberculosis.

113. The composition according to claim 51, wherein the polypeptide of (a) comprises an amino acid sequence at least 90% identical to SEQ ID NO:1.

114. The immunogenic composition according to claim 52, wherein the polypeptide of (a) comprises an amino acid sequence at least 90% identical to SEQ ID NO:1.

115. The method according to claim 60, wherein the polypeptide comprises an amino acid sequence at least 90% identical to SEQ ID NO:1.

116. The method according to claim 61, wherein the polynucleotide comprises a nucleic acid sequence at least 90% identical to SEQ ID NO:2.

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