



(51) Int.Cl.: **A 61 K 39/04 (2006.01)**  
**C 07 K 19/00 (2006.01)**

(45) Oversættelsen bekendtgjort den: **2019-03-25**

(80) Dato for Den Europæiske Patentmyndigheds  
bekendtgørelse om meddelelse af patentet: **2018-12-05**

(86) Europæisk ansøgning nr.: **10766664.6**

(86) Europæisk indleveringsdag: **2010-04-23**

(87) Den europæiske ansøgnings publiceringsdag: **2012-02-29**

(86) International ansøgning nr.: **DK2010000054**

(87) Internationalt publikationsnr.: **WO2010121618**

(30) Prioritet: **2009-04-24 DK 200900539**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR**

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(54) Benævnelse: **TB-TUBERKULOSEVACCINE TIL FOREBYGGELSE AF REAKTIVERING**

(56) Fremdragne publikationer:  
**EP-A1- 1 350 839**  
**WO-A1-2006/072787**  
**WO-A2-01/04151**  
**WO-A2-02/054072**  
**WO-A2-2004/006952**  
**WO-A2-2006/136162**  
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# DESCRIPTION

## Field of invention

**[0001]** The present invention discloses a vaccine that can be administered to latently infected individuals to prevent reactivation of latent tuberculosis infection caused by species of the tuberculosis complex microorganisms (*Mycobacterium tuberculosis*, *M.bovis*, *M.africanum*), by targetting constitutively expressed antigens such as ESAT6, CFP10 and other antigens from the ESX-1 secretion system.

## General Background

**[0002]** Human tuberculosis caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) is a severe global health problem, responsible for approx. 3 million deaths annually, according to the WHO. The worldwide incidence of new tuberculosis (TB) cases had been falling during the 1960s and 1970s but during recent decades this trend has markedly changed in part due to the advent of AIDS and the appearance of multidrug resistant strains of *M. tuberculosis*.

**[0003]** Organisms of the tuberculosis complex can cause a variety of diseases, but the commonest route of invasion is by inhalation of bacteria. This initiates an infection in the lung, which can ultimately spread to other parts of the body. Normally, this infection is restricted in growth by the immune system, so that the majority of infected individuals show few signs apart from cough and fever, which eventually abates. Approximately 30% of individuals are unable to contain the infection and they will develop primary disease, which in many cases will eventually prove fatal. However, it is believed that even those individuals who apparently control the infection remain infected, probably for the rest of their life. Certainly, individuals who have been healthy for years or even decades can suddenly develop tuberculosis, which has proven to be caused by the same organism they were infected with many years previously. *M. tuberculosis* and other organisms of the TB complex are unique in that the mycobacteria can evade the immune response and survive for long periods in a refractory non-replicating or slowly-replicating stage. This is referred to as latent TB and is at present a very significant global health problem which is estimated to affect approximately 1/3 of the worlds population (Anon., 2001).

**[0004]** The only vaccine presently available for clinical use is BCG, a vaccine whose efficacy remains a matter of controversy. Although BCG consistently performs well in animal models of primary infection, it has clearly failed to control the TB epidemic. Consistent with that, BCG vaccination appears to provide protection against pediatric TB (which is due to primary infection), while offering little or no protection against adult disease (which is often reactivation of latent infection acquired in childhood). It has also been shown that vaccination of individuals who are currently sensitized to mycobacteria or latently infected is ineffective.

**[0005]** The course of a *M. tuberculosis* infection runs essentially through 3 phases, as illustrated in figure 1. During the acute phase, the bacteria proliferate in the organs, until the immune response increases to the point at which it can control the infection whereupon the bacterial load peaks and starts declining. After this, a latent phase is established where the bacterial load is kept stable at a low level. In this phase it has been the current thinking that *M. tuberculosis* goes from active multiplication to a state of dormancy, essentially becoming non-replicating and remaining inside the granuloma.

**[0006]** However, recently it has become clear that even in the stage of infection characterized by constant low bacterial numbers at least part of the bacterial population remain in a state of active metabolism (Talaat AM et al. 2007). These bacteria therefore survive, maintain an active metabolism and replicate in the face of a strong immune response. In the infected individual there is therefore a balance between non-replicating bacteria (that may be very difficult for the immune system to detect as they are located intracellularly) and slowly replicating bacteria that have an active but changed expression profile in an attempt to adapt to the hostile environment encountered in the immune host. Bacteria in this stage are typically not targeted by most of the preventive vaccines that are currently under development in the TB field as exemplified by the lack of activity when classical preventive vaccines are given to latently infected experimental animals (Turner 2000).

**[0007]** In some cases, the balance is tilted in favour of the pathogen and the infection goes into the reactivation phase, where the bacteria start replicating rapidly and bacterial numbers in the infected individual increases. Bacteria that replicate in latently infected individuals under very strong immune pressure are the target for the vaccination strategy in the present invention. Bacteria in this latent infective stage are typically not targeted by most of the preventive vaccines that are currently under development in the TB field as exemplified by the lack of activity when preventive vaccines are given to latently infected experimental animals (Turner et al 2000). This is not surprising as it is now known that a strong host immune response results in the down regulation of many antigens such as the major preventive vaccine antigen Ag85 and PstS (Rogerson, BJ et al 2006). For Ag85B it has been shown that after infection there is an initial transient increase in Ag85B expression but already after two weeks infection the level of bacterial Ag85B expression had dropped from 0.3 transcripts per CFU of *M.tb.* during the peak period to 0.02 transcripts per CFU and this low level is maintained at least up to 100 days post infection. Thus at any time point after week 2 of infection less than 2 % of the bacteria actively express Ag85B (ibid.). The low expression of Ag85B is supported by a rapid drop in the number of T cells capable of making IFN- $\gamma$  in response to Ag85B in the lung 3 weeks post infection or later.

**[0008]** In contrast some antigens are more stably (constitutively) expressed throughout the different stages of infection and one example of this is ESAT6. After the initial infection phase the ESAT-6 expression level stabilizes at 0.8 transcripts per CFU *M. tuberculosis*. This is a transcription level much higher than for Ag85B and this level is maintained stably up to at least 100 days post infection (Rogerson, BJ et al 2006). Again transcription data is supported by

immune data that shows strong T cell recognition of ESAT-6 at the later stages of infection at the site of infection (ibid.). This constitutive expression pattern is an important feature that illustrates that these molecules fulfill essential functions of crucial importance for the pathogen, functions that depend upon genes that need to be constitutively expressed for the pathogen to survive in the immune host. These molecules are the basis for the current invention and are particularly important antigens for vaccines administered to latently infected individuals as they target all stages of the bacterial lifestyle and therefore have the broadest possible basis for activity. This is different from current thinking that has been focused on identifying the antigens upregulated by mycobacteria during non-replicating persistence (Andersen, P. 2007, WO02048391, WO04006952, Lin MY and Ottenhoff TH 2008; Leyten EM. et al. 2006). Although such antigens are upregulated during non-replicating persistence they may not always be available for immune recognition as the amounts available from non-replicating bacteria are below a reasonable threshold for detection or for the triggering of protective immune effector functions.

**[0009]** In contrast, several of the proteins from the ESX-1 secretion system have been shown to be highly immunogenic and expressed at high levels . ESX-1 is conserved in several pathogenic mycobacteria and involved in virulence of tubercle bacilli. The contribution of the individual ESX-1 proteins in secretion of ESAT-6, CFP10 and EspA has been well documented (Pym AS et al 2003; Guinn KI et al, 2004; Stanley, SA et al. 2003; Brodin, P. et al. 2006; MacGurn JA et al. 2005; Raghavan, S. et al. 2008) and the function of the effector molecules has been shown to be membrane lysis, escape from the phagosome and bacterial spreading (Gao LY et al 2004; Smith J. et al. 2008).

**[0010]** The full nature of the immune response that controls latent infection and the factors that lead to reactivation are largely unknown. However, there is some evidence for a shift in the dominant cell types responsible. While CD4 T cells are essential and sufficient for control of infection during the acute phase, studies suggest that CD8 T cell responses are more important in the latent phase (van Pinxteren LA et al. 2000).

**[0011]** As one skilled in the art will readily appreciate, expression of a gene is not sufficient to make it a good vaccine candidate. The only way to determine if a protein is recognized by the immune system during latent infection with *M. tuberculosis* is to produce the given protein and test it in an appropriate assay as described herein. In this regard, our group has demonstrated that antigens strongly expressed by mycobacteria, such as ESAT-6 (Early Secretory Antigen Target-6) are recognized in individuals in all stages of infection and in fact in particular in latently infected individuals (Boesen , Ravn, Doherty 2002). However the ESAT-6 specific T cells primed during the natural infection are, although they may be present in large numbers, almost exclusively of the so-called effector phenotype that are terminally differentiated T cells with a very limited lifespan and of low activity as protective T cells against infectious diseases (Seder R, et al. 2008). This is markedly different from the high quality, so-called polyfunctional T cells that are promoted by the vaccine demonstrated in the present study to protect against reactivation of TB.

It is far from all highly expressed and immunogenic proteins that are useful as post exposure

vaccines because many will provoke hypersensitivity reactions and thereby worsen the situation instead. This was clearly demonstrated in the clinical trial of Kock's original tuberculin vaccine. The vaccine was given as a post exposure vaccine to patients suffering from different forms of the disease including skin and pulmonary TB. The trial was a complete failure and several of the enrolled patients died because of severe hypersensitive reactions (Guttstadt A. 1891). Of the several hundred antigens known to be expressed during primary infection, and tested as vaccines, less than a half dozen have demonstrated significant potential. So far only one antigen has been shown to have any potential as a postexposure vaccine (Lowrie, 1999). However this vaccine only worked if given as a DNA vaccine, an experimental technique so far not approved for use in humans. Moreover, the technique has proved controversial, with other groups claiming that vaccination using this protocol induces either non-specific protection or even worsens disease (Turner, 2000).

**[0012]** WO 2004/006952 A2 discloses a therapeutic vaccine against latent or active tuberculosis infection, comprising a polypeptide antigen induced under low oxygen conditions.

**[0013]** Henao-Tamayo et al., *Tuberculosis* 89 (2009) 142-148, describe a post-exposure vaccination study in guinea pigs against *Mycobacterium tuberculosis*. It was concluded that the vaccination of this study could initially slow the disease process, but that the effect was transient.

**[0014]** Therefore, an effective postexposure vaccination strategy to protect infected individuals against reactivation of the disease is highly desireable.

### **Summary of the invention**

**[0015]** The invention is related to treating infections caused by species of the tuberculosis complex (*Mycobacterium tuberculosis*, *M. bovis*, *M. africanum*) by a vaccine that can be administered to latently infected individuals to prevent reactivation of latent tuberculosis infection caused by species of the tuberculosis complex microorganisms (*Mycobacterium tuberculosis*., *M.bovis*, *M.africanum*), by targetting constitutively expressed antigens such as ESAT6 and CFP10. ESAT6 and CFP10 are all interdependently required for secretion and all belong to the ESX-1 secretion system known to be essential for virulence. These secreted antigens are crucial for bacterial dessemination and lysis of cellular membranes. ESAT6 and CFP10 are also antigens that are constitutively expressed in the different stages of disease - whereas e.g. the expression of Ag85 is downregulated shortly after infection. Surprisingly, immunogenic constitutively expressed antigens prevent reactivation of latent tuberculosis infection when administered as a post exposure vaccine, thereby keeping the infection latent.

### **Detailed disclosure of the invention**

**[0016]** The invention discloses a vaccine or immunogenic composition that is administered postexposure to latently infected individuals that prevents reactivation of tuberculosis comprising an antigen which is constitutively expressed during infection with *M.tuberculosis* or a nucleic acid encoding said antigen.

**[0017]** The invention relates in particular to a vaccine for use in the prevention of reactivation of tuberculosis in individuals latently infected with *M.tuberculosis*, comprising an antigen which is constitutively expressed during infection with *M.tuberculosis* or a nucleic acid encoding said antigen,

wherein the antigen, belonging to the ESX-1 secretion system, is selected from the group consisting of

1. i) ESAT6, CFP10, Rv3614c, Rv3615c, EspR, Rv3868, Rv3869, Rv3870, Rv3871, Rv3872, Rv3873, Rv3876, Rv3877, Rv3878, Rv3879c, Rv3880c, Rv3881c, Rv3882c, Rv3883c and Rv3865,
2. ii) an immunogenic portion comprising an epitope for a B-cell or T-cell of any one of the sequences in (i); and
3. iii) an amino acid sequence analogue having at least 90% sequence identity to any one of the sequences in (i) or (ii) and at the same time being immunogenic by preventing reactivation of latent tuberculosis infection when administered as a therapeutic vaccine.

**[0018]** Preferably the composition comprises constitutively expressed antigens belonging to the ESX-1 secretion system, ESAT6, CFP10, Rv3614c, Rv3615c, EspR, Rv3868, Rv3869, Rv3870, Rv3871, Rv3872, Rv3873, Rv3876, Rv3877, Rv3878, Rv3879c, Rv3880c, Rv3881c, Rv3882c, Rv3883c, Rv3865c or an immunogenic portion, e.g. a T-cell epitope, of any one of these sequences or an amino acid sequence analogue having at least 90% sequence identity to any one of the sequences in and at the same time being immunogenic.

**[0019]** Also described herein is a composition that comprises a mix of immunogenic portions preferably selected from the group consisting of SEQ ID NO. 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 and 31.

**[0020]** Another embodiment of the invention is a composition where said polypeptides are fused to an antigen expressed by bacteria within the mycobacteria family, preferably where the fusion partner is a antigen which is constitutively expressed. A preferred fusion protein comprises ESAT6 fused to CFP10.

**[0021]** The composition according to the invention preferably comprises an additional delivery system selected among, live recombinant vaccines, that is gene-modified organisms such as bacteria or viruses expressing mycobacterial genes, or immunogenic delivery systems such as DNA vaccines, that is plasmids expressing genes or gene fragments for the proteins described above, or protein vaccines, that is the proteins themselves or synthetic peptides derived from the proteins themselves delivered in a delivery system such as an adjuvant. The adjuvant is

preferably selected from the group consisting of dimethyldiocta-decylammonium bromide (DDA), Quil A, poly I:C, aluminium hydroxide, Freund's incomplete adjuvant, IFN- $\gamma$ , IL-2, IL-12, monophosphoryl lipid A (MPL), Trehalose Dimycolate (TDM), Trehalose Dibehenate and muramyl dipeptide (MDP) most preferably an adjuvant promoting a polyfunctional T-cell response such as DDA/TDB and IC31.

The most preferred adjuvant comprises DDA/TDB and/or poly I:C. Alternatively the amino acid sequence is lipidated so as to allow a self-adjuvanting effect of the polypeptide.

**[0022]** The invention also discloses antigens described above for use in treatment of latent tuberculosis and preventing reactivation of the infection.

**[0023]** A method is also disclosed for treating an animal, including a human being, against reactivation of the tuberculosis infection caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, comprising administering to the animal the vaccine or immunogenic composition described above, wherein said vaccine or immunogenic composition is administered post infection, such as during or after acute stage infection and/or during latent stage infection.

**[0024]** The method can comprise a step of identifying a subject latently infected with a virulent mycobacteria e.g. by a diagnostic procedure such as the Mantoux tuberculin skin test (TST), the Quantiferon test, in vitro detection of responses to HBHA or the detection of IP10 after stimulation with a constitutively expressed antigen.

**[0025]** Also disclosed is the use of an antigen described above for the manufacture of a postexposure vaccine or immunogenic composition against reactivation of latent infections caused by species of the tuberculosis complex e.g. *Mycobacterium tuberculosis*, *M. bovis* and *M. africanum*, wherein said vaccine or immunogenic composition is for administration post infection, such as during or after acute stage infection and/or during latent stage comprising one or more immunogenic portions described above.

**[0026]** *Mycobacterium*'s success as a pathogen is due to the complex way it interacts with its host - a process controlled in part by the specialized ESX-1 bacterial protein-secretion system. The ESX-1 system delivers bacterial proteins (e.g. ESAT-6, CFP10 and EspA) into host cells and is critical for virulence. After being secreted from the bacilli the ESAT-6 protein forms pores in the phagosomal membrane, allowing the bacilli to escape into the cytosol from their containment in the phagosome and thereby it facilitates cell-to-cell spread.

**[0027]** The constitutive expression pattern is an important feature that illustrates that these molecules fulfill essential functions of crucial importance for the pathogen, functions that depend upon genes that need to be constitutively expressed for the pathogen to survive in the immune host. These molecules are the basis for the current invention and are particularly important antigens for vaccines administered to latently infected individuals as they target all stages of the bacterial lifestyle and therefore have the broadest possible basis for activity.

**[0028]** ESAT6, CFP10 and EspA are all interdependently required for secretion and all belong to the ESX-1 secretion system known to be essential for virulence. These secreted antigens are crucial for bacterial dissemination and lysis of cellular membranes. ESAT6, CFP10 and EspA are also antigens that are constitutively expressed in the different stages of disease - whereas e.g. the expression of Ag85 is downregulated shortly after infection. Immunogenic constitutively expressed antigens prevent reactivation of latent tuberculosis infection when administered as a therapeutic vaccine, thereby keeping the infection latent.

### Definitions

#### *Polyfunctional T cells*

**[0029]** By the term Polyfunctional T cells is understood T cells that simultaneously express all the cytokines IFN- $\gamma$ , IL-2, and TNF- $\alpha$ , or IL-2 plus at least one of the two other cytokines IFN- $\gamma$  and TNF- $\alpha$ .

#### *Polypeptides*

**[0030]** The word "polypeptide" in the present invention should have its usual meaning. That is an amino acid chain of any length, including a full-length protein, oligopeptides, short peptides and fragments thereof, wherein the amino acid residues are linked by covalent peptide bonds.

**[0031]** The polypeptide may be chemically modified by being glycosylated, by being lipidated (e.g. by chemical lipidation with palmitoyloxy succinimide as described by Mowat et al. 1991 or with dodecanoyl chloride as described by Lustig et al. 1976), by comprising prosthetic groups, or by containing additional amino acids such as e.g. a his-tag or a signal peptide.

**[0032]** Each polypeptide may thus be characterised by specific amino acids and be encoded by specific nucleic acid sequences. It will be understood that such sequences include analogues and variants produced by recombinant or synthetic methods wherein such polypeptide sequences have been modified by substitution, insertion, addition or deletion of one or more amino acid residues in the recombinant polypeptide and still be immunogenic in any of the biological assays described herein. Substitutions are preferably "conservative". These are defined according to the following table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other. The amino acids in the third column are indicated in one-letter code.

ALIPHATIC	Non-polar	GAP
		ILV
	Polar-uncharged	CSTM

	Polar-charged	NQ DE KR
AROMATIC		HFWY

**[0033]** A preferred polypeptide within the present invention is an immunogenic antigen from *M. tuberculosis* produced when the organism is subjected to the stresses associated with latent infection. Such antigen can for example also be derived from the *M. tuberculosis* cell and/or *M. tuberculosis* culture filtrate. Thus, a polypeptide comprising an immunogenic portion of one of the above antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *M. tuberculosis* antigen or be heterologous and such sequences may, but need not, be immunogenic.

**[0034]** Each polypeptide is encoded by a specific nucleic acid sequence. It will be understood that such sequences include analogues and variants hereof wherein such nucleic acid sequences have been modified by substitution, insertion, addition or deletion of one or more nucleic acid. Substitutions are preferably silent substitutions in the codon usage which will not lead to any change in the amino acid sequence, but may be introduced to enhance the expression of the protein.

**[0035]** In the present context the term "substantially pure polypeptide fragment" means a polypeptide preparation which contains at most 5% by weight of other polypeptide material with which it is natively associated (lower percentages of other polypeptide material are preferred, e.g. at most 4%, at most 3%, at most 2%, at most 1%, and at most ½%). It is preferred that the substantially pure polypeptide is at least 96% pure, *i.e.* that the polypeptide constitutes at least 96% by weight of total polypeptide material present in the preparation, and higher percentages are preferred, such as at least 97%, at least 98%, at least 99%, at least 99,25%, at least 99,5%, and at least 99,75%. It is especially preferred that the polypeptide fragment is in "essentially pure form", *i.e.* that the polypeptide fragment is essentially free of any other antigen with which it is natively associated, *i.e.* free of any other antigen from bacteria belonging to the tuberculosis complex or a virulent mycobacterium. This can be accomplished by preparing the polypeptide fragment by means of recombinant methods in a non-mycobacterial host cell as will be described in detail below, or by synthesizing the polypeptide fragment by the well-known methods of solid or liquid phase peptide synthesis, e.g. by the method described by Merrifield or variations thereof. For the purpose of the present invention it will be understood that the above definition of "substantially pure polypeptide or polypeptide fragment" does not exclude such polypeptides or polypeptide fragments when present in combination with other purified or synthetic antigens of mycobacterial or non-mycobacterial origin.

**[0036]** By the term "virulent mycobacterium" is understood a bacterium capable of causing the tuberculosis disease in an animal or in a human being. Examples of virulent mycobacteria

include but are not limited to *M. tuberculosis*, *M. africanum*, and *M. bovis*. Examples of relevant animals are cattle, possums, badgers and kangaroos.

**[0037]** By "an infected individual" is understood an individual with culture or microscopically proven infection with virulent mycobacteria, and/or an individual clinically diagnosed with TB and who is responsive to anti-TB chemotherapy. Culture, microscopy and clinical diagnosis of TB are well known by any person skilled in the art.

**[0038]** By the term "PPD-positive individual" is understood an individual with a positive Mantoux test or an individual where PPD (purified protein derivative) induces a positive *in vitro* recall response determined by release of IFN- $\gamma$ .

**[0039]** By "a latently infected individual" is understood an individual who has been infected by a virulent mycobacterium, e.g. *M. tuberculosis*, but shows no sign of active tuberculosis. It is likely that individuals who have been vaccinated, e.g. by BCG, or treated for TB may still retain the mycobacteria within their bodies, although this is currently impossible to prove since such individuals would be expected to be positive if tested for PPD reactivity. Nonetheless, in its most accurate sense, "latently-infected" may be used to describe any individual who has *M. tuberculosis* residing in their tissues but who is not clinically ill. A latently infected individual can be identified by a number of methods in clinical use today such as the Mantoux tuberculin skin test (TST), the Quantiferon test and in the future there may be even more sensitive means of diagnosing this particular stage of the infection such as the recently suggested *in vitro* detection of responses to HBHA (Hougaard 2007) or the detection of IP10 after stimulation *in vitro* with ESAT6 (Ruhwald 2008)

**[0040]** By the term "reactivation" is understood the situation where the balance between non-replicating bacteria (that may be very difficult for the immune system to detect as they are located intracellularly) and slowly replicating bacteria that has an active but changed expression profile in an attempt to adapt to the hostile environment encountered in the immune host is tilted in favour of the pathogen and the infection goes into the phase where the bacteria start replicating rapidly again and bacterial numbers in the infected individual increase. These bacteria that replicate in latently infected individuals under very strong immune pressure are the target for the vaccination strategy in the present invention.

**[0041]** By the term "IFN- $\gamma$ " is understood interferon-gamma. The measurement of IFN- $\gamma$  is used as an indication of an immunological response.

**[0042]** By the terms "nucleic acid fragment" and "nucleic acid sequence" are understood any nucleic acid molecule including DNA, RNA, LNA (locked nucleic acids), PNA, RNA, dsRNA and RNA-DNA-hybrids. Also included are nucleic acid molecules comprising non-naturally occurring nucleosides. The term includes nucleic acid molecules of any length e.g. from 10 to 10000 nucleotides, depending on the use. When the nucleic acid molecule is for use as a pharmaceutical, e.g. in DNA therapy, or for use in a method for producing a polypeptide according to the invention, a molecule encoding at least one epitope is preferably used, having

a length from about 18 to about 1000 nucleotides, the molecule being optionally inserted into a vector.

**[0043]** Throughout this specification, unless the context requires otherwise, the word "comprise", or variations thereof such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

**[0044]** Constitutively expressed genes are defined as genes that after a detailed analysis of mRNA at a population level are equally well expressed in vivo in the lung at time points later than three weeks post infection after being correlated for *M. tb* CFU numbers in the lung. From this definition it follows that a constitutive gene may be differentially expressed at a single bacteria level. The method to quantitate gene expression is quantitative PCR. "Equally well" is defined as being within +/- 5 fold the level from the previous measurement. The comparison is always to the time point immediately preceding the current. Time between measurements cannot be longer than the time between infection and the previous measurement. E.g. if expression of a gene is measured the first time at week 3 post infection the second measurement cannot be done later than 6 weeks post infection and the third 12 weeks post infection etc.

**[0045]** Constitutively expressed antigens are polypeptides or part of these polypeptides which are products of constitutively expressed genes.

#### ***Sequence identity***

**[0046]** The term "sequence identity" indicates a quantitative measure of the degree of homology between two amino acid sequences of equal length or between two nucleotide sequences of equal length. The two sequences to be compared must be aligned to best possible fit allowing the insertion of gaps or alternatively, truncation at the ends of the protein sequences. The sequence identity can be calculated as

$$\frac{(N_{ref} - N_{dif})}{N_{ref}} \cdot 100$$

wherein  $N_{dif}$  is the total number of non-identical residues in the two sequences when aligned and wherein  $N_{ref}$  is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC ( $N_{dif}=2$  and  $N_{ref}=8$ ). A gap is counted as non-identity of the specific residue(s), i.e. the DNA sequence AGTGTC will have a sequence identity of 75% with the DNA sequence AGTCAGTC ( $N_{dif}=2$  and  $N_{ref}=8$ ). Sequence identity can alternatively be calculated by the BLAST program e.g. the BLASTP program (Pearson, 1988, or [www.ncbi.nlm.nih.gov/cgi-bin/BLAST](http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST)). In one aspect of the invention, alignment is performed with the sequence alignment method Clus-talW with default parameters as described by Thompson J., et al 1994, available at <http://www2.ebi.ac.uk/clustalw/>.

**[0047]** A preferred minimum percentage of sequence identity is at least 80%, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, and at least 99.5%.

*Immunogenic portion*

**[0048]** In a preferred embodiment of the invention, the polypeptide comprises an immunogenic portion of the polypeptide, such as an epitope for a B-cell or T-cell.

The immunogenic portion of a polypeptide is a part of the polypeptide which elicits an immune response in an animal or a human being, and/or in a biological sample determined by any of the biological assays described herein. The immunogenic portion of a polypeptide may be a T-cell epitope or a B-cell epitope. Immunogenic portions can be related to one or a few relatively small parts of the polypeptide, they can be scattered throughout the polypeptide sequence or be situated in specific parts of the polypeptide. For a few polypeptides epitopes have even been demonstrated to be scattered throughout the polypeptide covering the full sequence (Ravn et al 1999). In order to identify relevant T-cell epitopes which are recognised during an immune response, it is possible to use overlapping oligopeptides for the detection of MHC class II epitopes, preferably synthetic, having a length of e.g. 20 amino acid residues derived from the polypeptide. These peptides can be tested in biological assays (e.g. the IFN- $\gamma$  assay as described herein) and some of these will give a positive response (and thereby be immunogenic) as evidence for the presence of a T cell epitope in the peptide. For ESAT-6 and CFP10 such studies have shown that every part of the antigen contains T-cell epitopes (Mustafa et al. 2000, Arend SM et al. 2000). For the detection of MHC class I epitopes it is possible to predict peptides that will bind (Stryhn et al. 1996) and hereafter produce these peptides synthetically and test them in relevant biological assays e.g. the IFN- $\gamma$  assay as described herein. The peptides preferably having a length of e.g. 8 to 11 amino acid residues derived from the polypeptide. B-cell epitopes can be determined by analysing the B cell recognition to overlapping peptides covering the polypeptide of interest as e.g. described in Harboe et al 1998. Consistent with this definition, an immunogenic portion of a polypeptide as described herein can be identified as a portion which elicits an immune response, c.f. the definition of "immune response" herein below.

**[0049]** Although the minimum length of a T-cell epitope has been shown to be at least 6 amino acids, it is normal that such epitopes are constituted of longer stretches of amino acids. Hence, it is preferred that the polypeptide fragment of the invention has a length of at least 7 amino acid residues, such as at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 22, at least 24, and at least 30 amino acid residues. Hence, in important embodiments of the inventive method, it is preferred that the polypeptide fragment has a length of at most 50 amino acid residues, such as at most 40, 35, 30, 25, and 20 amino acid residues. It is expected that the peptides having a length of between 10 and 20 amino acid residues will prove to be most efficient as MHC class II epitopes and therefore especially preferred lengths of the polypeptide fragment used in the inventive method are 18, such as 15, 14, 13, 12 and even 11 amino acid residues. It is expected that the peptides having a length of

between 7 and 12 amino acid residues will prove to be most efficient as MHC class I epitopes and therefore especially preferred lengths of the polypeptide fragment used in the inventive method are 11, such as 10, 9, 8 and even 7 amino acid residues.

**[0050]** Immunogenic portions of polypeptides may be recognised by a broad part (high frequency) or by a minor part (low frequency) of the genetically heterogeneous human population. In addition some immunogenic portions induce high immunological responses (dominant), whereas others induce lower, but still significant, responses (subdominant). High frequency><low frequency can be related to the immunogenic portion binding to widely distributed MHC molecules (HLA type) or even by multiple MHC molecules (Sinigaglia, 1988, Kilgus, 1991).

**[0051]** In the context of providing candidate molecules for a new vaccine against tuberculosis, the subdominant epitopes are however as relevant as are the dominant epitopes since it has been shown (Olsen, 2000) that such epitopes can induce protection regardless of the fact that they are not as strongly or broadly recognised.

#### ***Variants***

**[0052]** A common feature of the polypeptides of the invention is their capability to induce an immunological response as illustrated in the examples. It is understood that a variant of a polypeptide of the invention produced by substitution, insertion, addition or deletion may also be immunogenic as determined by any of the assays described herein.

#### ***Immune individual***

**[0053]** An immune individual is defined as a person or an animal, which has cleared or controlled an infection with virulent mycobacteria or has received a prophylactic vaccination, such as vaccination with *M. bovis* BCG.

#### ***Immune response***

**[0054]** The immune response may be monitored by one of the following methods:

- An in vitro cellular response is determined by induction of the release of a relevant cytokine such as IFN- $\gamma$  from, or the induction of proliferation in lymphocytes withdrawn from an animal or human being currently or previously infected with virulent mycobacteria or immunized with the relevant polypeptide. The induction being performed by the addition of the polypeptide or the immunogenic portion of the polypeptide to a suspension comprising from  $2 \times 10^5$  cells to  $4 \times 10^5$  cells per well. The cells being isolated

from either the blood, the spleen, the liver or the lung and the addition of the polypeptide or the immunogenic portion resulting in a concentration of not more than 20 µg per ml suspension and the stimulation being performed from two to five days. For monitoring cell proliferation the cells are pulsed with radioactive labeled Thymidine and after 16-22 hours of incubation detecting the proliferation by liquid scintillation counting. A positive response is defined as being a response more than background plus two standard deviations. The release of IFN-γ can be determined by the ELISA method, which is well known to a person skilled in the art. A positive response being a response more than background plus two standard deviations. Other cytokines than IFN-γ could be relevant when monitoring the immunological response to the polypeptide, such as IL-12, TNF-α, IL-4, IL-5, IL-10, IL-6, TGF-β. Another and more sensitive method for detecting the immune response is the ELISpot method, in which the frequency of IFN-γ producing cells is determined. In an ELISpot plate (MAHA, Millipore) precoated with anti-murine IFN-γ antibodies (PharMingen) graded numbers of cells isolated from either blood, spleen, or lung (typically between 1 to 4 x 10<sup>5</sup> cells /well) are incubated for 24-32 hrs in the presence of the polypeptide or the immunogenic portion resulting in a concentration of not more than 20 µg per ml. The plates are subsequently incubated with biotinylated anti-IFN-γ antibodies followed by a streptavidin-alkaline phosphatase incubation. The IFN-γ producing cells are identified by adding BCIP/NBT (Sigma), the relevant substrate giving rise to spots. These spots can be enumerated using a dissection microscope. It is also a possibility to determine the presence of mRNA coding for the relevant cytokine by the use of the PCR technique. Usually one or more cytokines will be measured utilizing for example PCR, ELISPOT or ELISA. It will be appreciated by a person skilled in the art that a significant increase or decrease in the amount of any of these cytokines induced by a specific polypeptide can be used in evaluation of the immunological activity of the polypeptide.

- An *in vitro* cellular response may also be determined by the use of T cell lines derived from an immune individual or an *M. tuberculosis*-infected person where the T cell lines have been driven with either live mycobacteria, extracts from the bacterial cell or culture filtrate for 10 to 20 days with the addition of IL-2. The induction being performed by addition of not more than 20 µg polypeptide per ml suspension to the T cell lines containing from 1x10<sup>5</sup> cells to 3x10<sup>5</sup> cells per well and incubation being performed from two to six days. The induction of IFN-γ or release of another relevant cytokine is detected by ELISA. The stimulation of T cells can also be monitored by detecting cell proliferation using radioactively labeled Thymidine as described above. For both assays a positive response being a response more than background plus two standard deviations.
- An *in vivo* cellular response may be determined as a positive DTH response after intradermal injection or local application patch of at most 100 µg of the polypeptide or the immunogenic portion to an individual who is clinically or subclinically infected with a virulent mycobacterium, a positive response having a diameter of at least 5 mm 72-96 hours after the injection or application.
- An *in vitro* humoral response is determined by a specific antibody response in an immune or infected individual. The presence of antibodies may be determined by an

ELISA technique or a Western blot where the polypeptide or the immunogenic portion is absorbed to either a nitrocellulose membrane or a polystyrene surface. The serum is preferably diluted in PBS from 1:10 to 1:100 and added to the absorbed polypeptide and the incubation being performed from 1 to 12 hours. By the use of labeled secondary antibodies the presence of specific antibodies can be determined by measuring the OD e.g. by ELISA where a positive response is a response of more than background plus two standard deviations or alternatively a visual response in a Western blot.

- Another relevant parameter is measurement of the protection in animal models induced after vaccination with the polypeptide in an adjuvant or after DNA vaccination. Suitable animal models include primates, guinea pigs or mice, which are challenged with an infection of a virulent Mycobacterium. Readout for induced protection could be decrease of the bacterial load in target organs compared to non-vaccinated animals, prolonged survival times compared to non-vaccinated animals and diminished weight loss compared to non-vaccinated animals.

### ***Preparation methods***

**[0055]** In general, *M. tuberculosis* antigens, and DNA sequences encoding such antigens, may be prepared using any one of a variety of procedures. They may be purified as native proteins from the *M. tuberculosis* cell or culture filtrate by procedures such as those described above. Immunogenic antigens may also be produced recombinantly using a DNA sequence encoding the antigen, which has been inserted into an expression vector and expressed in an appropriate host. Examples of host cells are *E. coli*. The polypeptides or immunogenic portion hereof can also be produced synthetically having fewer than about 100 amino acids, and generally fewer than 50 amino acids and may be generated using techniques well known to those ordinarily skilled in the art, such as commercially available solid-phase techniques where amino acids are sequentially added to a growing amino acid chain.

**[0056]** In the construction and preparation of plasmid DNA encoding the polypeptide as defined for DNA vaccination a host strain such as *E. coli* can be used. Plasmid DNA can then be prepared from cultures of the host strain carrying the plasmid of interest, and purified using e.g. the Qiagen Giga -Plasmid column kit (Qiagen, Santa Clarita, CA, USA) including an endotoxin removal step. It is preferred that plasmid DNA used for DNA vaccination is endotoxin free.

### ***Fusion proteins***

**[0057]** The immunogenic polypeptides may also be produced as fusion proteins, by which methods superior characteristics of the polypeptide of the invention can be achieved. For instance, fusion partners that facilitate export of the polypeptide when produced recombinantly,

fusion partners that facilitate purification of the polypeptide, and fusion partners which enhance the immunogenicity of the polypeptide fragment of the invention are all interesting possibilities. Therefore, the invention also pertains to a fusion polypeptide comprising at least one polypeptide or immunogenic portion defined above and at least one fusion partner. The fusion partner can, in order to enhance immunogenicity, be another polypeptide derived from *M. tuberculosis*, such as of a polypeptide fragment derived from a bacterium belonging to the tuberculosis complex, such as ESAT-6, CFP10, TB10.4, RD1-ORF5, RD1-ORF2, Rv1036, MPB64, MPT64, Ag85A, Ag85B (MPT59), MPB59, Ag85C, 19kDa lipoprotein, MPT32 and alpha-crystallin, or at least one T-cell epitope of any of the above mentioned antigens (Skjøt et al 2000; WO0179274; WO01 04151; US patent application 09/0505,739; Rosenkrands et al 1998; Nagai et al 1991). The invention also pertains to a fusion polypeptide comprising mutual fusions of two or more of the polypeptides (or immunogenic portions thereof) of the invention. Other fusion partners, which could enhance the immunogenicity of the product, are lymphokines such as IFN- $\gamma$ , IL-2 and IL-12. In order to facilitate expression and/or purification, the fusion partner can e.g. be a bacterial fimbrial protein, e.g. the pilus components pilin and papA; protein A; the ZZ-peptide (ZZ-fusions are marketed by Pharmacia in Sweden); the maltose binding protein; glutathione S-transferase;  $\beta$ -galactosidase; or poly-histidine. Fusion proteins can be produced recombinantly in a host cell, which could be *E. coli*, and it is a possibility to induce a linker region between the different fusion partners.

**[0058]** Other interesting fusion partners are polypeptides, which are lipidated so that the immunogenic polypeptide is presented in a suitable manner to the immune system. This effect is e.g. known from vaccines based on the *Borrelia burgdorferi* OspA polypeptide as described in e.g. WO 96/40718 A or vaccines based on the *Pseudomonas aeruginosa* Oprl lipoprotein (Cote-Sierra J 1998). Another possibility is N-terminal fusion of a known signal sequence and an N-terminal cystein to the immunogenic polypeptide. Such a fusion results in lipidation of the immunogenic polypeptide at the N-terminal cystein, when produced in a suitable production host.

### **Uses**

#### **Vaccine**

**[0059]** A vaccine is a biological preparation that establishes or improves immunity to a particular disease. Vaccines can be prophylactic (e.g. to prevent or ameliorate the effects of a future infection by any natural or "wild" pathogen), postexposure (e.g. to prevent reactivation in latently infected individuals without clinical symptoms) or therapeutic (e.g. vaccines used to treat active disease either alone or combined with antibiotic treatment to shorten treatment)

#### **An animal model for latent TB**

**[0060]** To induce a low grade latent infection with *M.tb*, animals are first given an aerosol infection using a normal dose of *M.tb* (approximately 150 bacteria in the lungs). After 6 weeks of infection, the animals are then given a suboptimal chemotherapy treatment of 6 weeks during which most - but not all - of the bacteria are eradicated. The remaining bacteria will establish a latent infection. Following the chemotherapy treatment some animals will be vaccinated to examine the ability of the vaccine to prevent re-activation of the latent infection, which will occur spontaneously 5-15 weeks after the chemotherapy treatment. See figure 2.

***Protein Vaccine***

**[0061]** Another part of the invention pertains to a vaccine composition comprising a polypeptide (or at least one immunogenic portion thereof) or fusion polypeptide according to the invention. In order to ensure optimum performance of such a vaccine composition it is preferred that it comprises an immunologically and pharmaceutically acceptable carrier, vehicle or adjuvant.

**[0062]** An effective vaccine, wherein a polypeptide of the invention is recognized by the animal, will in an animal model be able to decrease bacterial load in target organs, prolong survival times and/or diminish weight loss after challenge with a virulent Mycobacterium, compared to non-vaccinated animals

**[0063]** Suitable carriers are selected from the group consisting of a polymer to which the polypeptide(s) is/are bound by hydrophobic non-covalent interaction, such as a plastic, e.g. polystyrene, or a polymer to which the polypeptide(s) is/are covalently bound, such as a polysaccharide, or a polypeptide, e.g. bovine serum albumin, ovalbumin or keyhole limpet haemocyanin. Suitable vehicles are selected from the group consisting of a diluent and a suspending agent. The adjuvant is preferably selected from the group consisting of dimethyldioctadecylammonium bromide (DDA), Quil A, poly I:C, aluminium hydroxide, Freund's incomplete adjuvant, IFN- $\gamma$ , IL-2, IL-12, monophosphoryl lipid A (MPL), Trehalose Dimycolate (TDM), Trehalose Dibehenate and muramyl dipeptide (MDP).

**[0064]** Preparation of vaccines which contain peptide sequences as active ingredients is generally well understood in the art, as exemplified by U.S. Patents 4,608,251; 4,601,903; 4,599,231 and 4,599,230.

**[0065]** Other methods of achieving adjuvant effect for the vaccine include use of agents such as aluminum hydroxide or phosphate (alum), synthetic polymers of sugars (Carbopol), aggregation of the protein in the vaccine by heat treatment, aggregation by reactivating with pepsin treated (Fab) antibodies to albumin, mixture with bacterial cells such as *C. parvum* or endotoxins or lipopolysaccharide components of gram-negative bacteria, emulsion in physiologically acceptable oil vehicles such as mannide mono-oleate (Aracel A) or emulsion with 20 percent solution of a perfluorocarbon (Fluosol-DA) used as a block substitute may also

be employed. Other possibilities involve the use of immune modulating substances such as cytokines or synthetic IFN- $\gamma$  inducers such as poly I:C in combination with the above-mentioned adjuvants.

**[0066]** Another interesting possibility for achieving adjuvant effect is to employ the technique described in Gosselin *et al.*, 1992. In brief, a relevant antigen such as an antigen of the present invention can be conjugated to an antibody (or antigen binding antibody fragment) against the Fc $\gamma$  receptors on monocytes/macrophages.

**[0067]** The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be immunogenic and effective in preventing re-activation. The quantity to be administered depends on the subject to be treated, including, e.g., the capacity of the individual's immune system to mount an immune response, and the degree of protection desired. Suitable dosage ranges are of the order of several hundred micrograms active ingredient per vaccination with a preferred range from about 0.1  $\mu$ g to 1000  $\mu$ g, such as in the range from about 1  $\mu$ g to 300  $\mu$ g, and especially in the range from about 10  $\mu$ g to 50  $\mu$ g. Suitable regimens for initial administration and booster shots are also variable but are typified by an initial administration followed by subsequent inoculations or other administrations.

**[0068]** The manner of application may be varied widely. Any of the conventional methods for administration of a vaccine are applicable. These are believed to include oral application on a solid physiologically acceptable base or in a physiologically acceptable dispersion, parenterally, by injection or the like. The dosage of the vaccine will depend on the route of administration and will vary according to the age of the person to be vaccinated and, to a lesser degree, the size of the person to be vaccinated.

**[0069]** The vaccines are conventionally administered intra pulmonary, e.g. by aerosol or inhalation, parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and advantageously contain 10-95% of active ingredient, preferably 25-70%.

**[0070]** In many instances, it will be necessary to have multiple administrations of the vaccine. In instances where the individual has already become infected or is suspected to have become infected, the previous vaccination may have provided sufficient immunity to prevent primary disease, but as discussed previously, boosting this immune response will not help against the latent infection. In such a situation, the vaccine will necessarily have to be a post exposure vaccine designed for efficacy against the latent stage of infection or reemerging active

tuberculosis infection.

**[0071]** Due to genetic variation, different individuals may react with immune responses of varying strength to the same polypeptide. Therefore, the vaccine according to the invention may comprise several different polypeptides in order to increase the immune response. The vaccine may comprise two or more polypeptides or immunogenic portions, where all of the polypeptides are as defined above, or some but not all of the peptides may be derived from virulent mycobacteria. In the latter example, the polypeptides not necessarily fulfilling the criteria set forth above for polypeptides may either act due to their own immunogenicity or merely act as adjuvants.

**[0072]** The vaccine may comprise 1-20, such as 2-20 or even 3-20 different polypeptides or fusion polypeptides, such as 3-10 different polypeptides or fusion polypeptides.

#### ***DNA Vaccine.***

**[0073]** The nucleic acid fragments of the invention may be used for effecting *in vivo* expression of antigens, *i.e.* the nucleic acid fragments may be used in so-called DNA vaccines as reviewed in Ulmer et al 1993.

**[0074]** Hence, also described herein to a post exposure vaccine comprising a nucleic acid fragment according to the invention, the vaccine effecting *in vivo* expression of antigen by an animal, including a human being, to whom the vaccine has been administered, the amount of expressed antigen being effective to confer treatment of the infections caused by virulent mycobacteria in an animal, including a human being.

**[0075]** The efficacy of such a DNA vaccine can possibly be enhanced by administering the gene encoding the expression product together with a DNA fragment encoding a polypeptide which has the capability of modulating an immune response.

#### ***Live recombinant vaccines***

**[0076]** One possibility for effectively activating a cellular immune response for a post exposure vaccine can be achieved by expressing the relevant antigen in a vaccine in a non-pathogenic microorganism or virus. Well-known examples of such microorganisms are *Mycobacterium bovis* BCG, *Salmonella* and *Pseudomonas* and examples of viruses are *Vaccinia* virus and *Adenovirus*.

**[0077]** Therefore, also described herein is an improvement of the living BCG vaccine presently available, wherein one or more copies of a DNA sequence encoding one or more polypeptide as defined above has been incorporated into the genome of the micro-organism in a manner

allowing the micro-organism to express and secrete the polypeptide. The incorporation of more than one copy of a nucleotide sequence of the invention is contemplated to enhance the immune response.

**[0078]** Another possibility is to integrate the DNA encoding the polypeptide according to the invention in an attenuated virus such as the vaccinia virus or Adenovirus (Rolph et al 1997). The recombinant vaccinia virus is able to replicate within the cytoplasma of the infected host cell and the polypeptide of interest can therefore induce an immune response, which is envisioned to induce protection against TB.

**[0079]** The invention will now be described in further details in the following non-limiting examples.

### Figure legends

#### **[0080]**

Figure 1: The course of an *M. tuberculosis* infection runs essentially through 3 phases

Figure 2: Model for postexposure vaccination to prevent reactivation

Figure 3: TB vaccination model.

A schematic overview of the model used at the SSI for the testing of postexposure vaccines. Mice are infected with virulent *M.tb* by the aerosol route. From week 6 to week 12 post infection mice are treated with antibiotics to establish a state of latent TB. The mice are vaccinated 2 to three times at 3 week intervals initiated at week 10 post infection with the postexposure vaccine candidates. The mice are allowed time to reactivate the disease and approximately 20 weeks later the lungs are assessed for bacterial numbers to assess the protective efficacy of the vaccine.

Figure 4: Post-exposure vaccine induced protection by ESAT6 but not Ag85.

Mice were infected, treated and vaccinated according to the schematic overview in example 1. Mice were killed between week 30-40 post infection and at this timepoint lungs were assessed for bacterial load (Figure A, C-E) or as displayed in figure 4B where the bacterial load was determined at several timepoints throughout infection for ESAT6. (A and B) Bacterial load of ESAT6 vaccinated compared to control animals. (C) Bacterial load of Ag85B vaccinated compared to control animals. (D) Bacterial load of ESAT-6 pepmix vaccinated (pool of overlapping peptides covering the entire ESAT6 sequence) compared to both Ag85B vaccinated and control animals. (E) Protection against reactivation following postexposure vaccination with Ag85B-ESAT-6 (H1) vaccinated compared to non-vaccinated control mice. All data in figure 4A, C-E are displayed as dot plots representing each individual animal with the mean depicted whereas each timepoint in figure 4B is representative of 6 individual animals and displayed as mean  $\pm$  standard error of the mean (SEM) (B). All statistical analyses were performed using either an unpaired t-test (Figure A-C and E) or Tukey's multiple comparison test (figure D)

where  $p<0.05$  was considered significant.

Figure 5: ESAT-6 postexposure vaccination induce polyfunctional T cells.

Cells from infected lungs from non-vaccinated or ESAT-6 vaccinated animals were stimulated in vitro with ESAT-6 prior to staining with anti-CD4, -CD8, -IFN- $\gamma$ , -TNF- $\alpha$  and -IL-2. (A and B) Cytokine profiles were determined by first dividing the CD4 T cells into IFN- $\gamma$  positive (+) or IFN- $\gamma$  negative (-) cells. Both the IFN- $\gamma$ + and IFN- $\gamma$ - cells were analyzed with respect to the production of TNF- $\alpha$  and IL-2. The pie charts (A and B) are colour coded according to the cytokine production profile and summarize the fractions of the CD4 $^{+}$  T cell response (out of the ESAT-6 specific CD4 T cells) that are positive for a given cytokine production profile. (C) Every possible combination of cytokines is shown on the x-axis of the bar chart and the percentage of ESAT-6 specific CD4 $^{+}$  T cells in non vaccinated mice (grey bars) or ESAT-6 vaccinated mice (Black bars) expressing any combination of cytokines is given for each immunization group. D. Latently infected mice were vaccinated twice with ESAT-6, and 20 weeks after the last vaccination, lungs were assessed for bacterial number to determine protective efficacy. (\*\* $p<0.01$ , One way ANOVA Tukey's multiple comparisons test).

Figure 6: Pooled analysis of all postexposure experiments

For an individual experiment where either ESAT6, Rv3871, Ag85B, Rv3905, Rv3445, Rv0569 or Rv2031c (Figure A), Ag85B-ESAT6 (H1) or Ag85B-ESAT6-Rv2660 (H56) (Figure B) was used for post-exposure vaccination the median of the bacterial load of the adjuvant control group was compared to the bacterial load of each individual mouse in a vaccinated group vaccinated with either one of the antigens mentioned above. In figure A and B each dot corresponds to the level of protection i.e.  $\Delta\text{Log}_{10}$  CFU conferred by the vaccination compared to the adjuvant control group and consists of several independent experiments. (A) Log10 protection for the single antigens ESAT6, Rv3871, Ag85B, Rv3905, Rv3445, Rv0569 or Rv2031c (B) or for the hybrid antigens H1 and H56 compared to ESAT6 alone. A statistical analysis was applied for comparisons of medians between the different groups either using the Kruskall Wallis multiple comparison test.  $p<0.05$  was considered significant.

Figure 7: Effect of postexposure vaccination with Rv3871 compared to ESAT6 and control animals.

Mice were infected, treated and vaccinated at week 10, 13 and 18 post infection. At week 36 post infection the mice were terminated and lung lymphocytes from both vaccinated and non-vaccinated saline control mice were restimulated in vitro with Rv3871 (Fig.7A) or ESAT6 (Fig.7B). IFN- $\gamma$  releases assessed by ELISA and samples were performed in triplicate. Data are depicted as mean  $\pm$  SEM. The protective efficacy conferred by the vaccines was determined by enumeration of bacteria in the lung cultured from full lung homogenate ( $n=16-18$ ). Data displayed as a dot plot where each dot represents an individual animal and depicted with the median (red line).

## Examples

**EXAMPLE 1: Murine TB model for vaccination**

**[0081]** The Cornell model has widely been used as a murine model for the study of latent TB. This model has been adapted in our laboratory for the testing of the ability of vaccine candidates to prevent reactivation. Mice are initially aerosolically infected with virulent *M.tb.* and at week 6 post infection antibiotic treatment is initiated to reduce the bacterial load. This is to mimic the latent stage of a human infection which does not occur spontaneously in mice. During this latent stage (a stage with continuous low bacterial numbers) the mice are being immunized twice and the ability to prevent reactivation by the vaccine is determined by culturing the spleen and lungs for live *M.tb.* 20 weeks after the last immunization. The long timespan of the experiments is necessary to allow sufficient time for reactivation of the disease which is a prerequisite for readout of vaccine efficacy (Figure 3).

**EXAMPLE 2 : Postexposure vaccine induced protection by ESAT6 but not Ag85.**

**[0082]** ESAT-6 and Ag85B have proven to be protective in prophylactic vaccination both as single components and also as the fusion molecule Ag85B-ESAT6 (H1). However, when these antigens were tested in the postexposure model (as described above in example 1) only ESAT6 has a protective effect and controls bacteria growth during the reactivation phase (Figure 4). Furthermore, as seen in figure 4B ESAT6 protection against reactivation manifests itself as early as W18 post infection and this protection was maintained throughout the course of the experiment (up until week 40 post infection). This is in contrast to what is observed when Ag85B is used as a post exposure vaccine (Figure 4C and D), where there is no significant decrease in bacterial load compared to the control. In addition, we evaluated the H1 fusion protein which is composed of the TB antigens Ag85B and ESAT-6 which has shown promising efficacy in a prophylactic setting. When this molecule was used as a post exposure vaccine in the SSI postexposure model it was able to significantly reduce the bacterial numbers (Figure 4E).

**EXAMPLE 3: Post exposure vaccine induced protection by ESAT6 peptide mix**

**[0083]** As shown in the examples above, the ESAT-6 molecule is very active when given post-exposure, resulting in a decrease in bacterial load compared to the control group and also compared to Ag85B. Furthermore we have shown that ESAT-6 given as a pool of overlapping peptides instead of a recombinant protein also leads to a better protection against re-activation compared to both the control group and Ag85B, demonstrating the strong activity of ESAT6, and ability to function as a post exposure vaccine (Figure 4D).

**[0084]** Overlapping ESAT-6 peptides (P1-P13) used for protection experiment:

P1 MTEQQWNFAGIEAAA (SEQ ID NO. 19)

P2 NFAGIEAAASAIQGN (SEQ ID NO. 20)

P3 ASAIQGNVTSIHSLL (SEQ ID NO. 21)

P4 NVTSIHSLLDEGKQS (SEQ ID NO. 22)

P5 SLLDEGKQSLTKLAA (SEQ ID NO. 23)

P6 KQSLTKLAAAWGGSG (SEQ ID NO. 24)

P7 AAWGGSGSEAYQGVQ (SEQ ID NO. 25)

P8 GSEAYQGVQQKWDAT (SEQ ID NO. 26)

P9 QQKWDATATELNNAL (SEQ ID NO. 27)

P10 TATELNNALQNLART (SEQ ID NO. 28)

P11 ALQNLARTISEAGQA (SEQ ID NO. 29)

P12 TISEAGQAMASTEGN (SEQ ID NO. 30)

P13 QAMASTEGNVTGMFA (SEQ ID NO. 31)

**EXAMPLE 5: Post exposure vaccination with ESAT-6 induced polyfunctional T cells**

**[0085]** To examine the effect of a post exposure vaccination with ESAT-6 on the cytokine expression profile of the ESAT-6 specific cells, mice were first aerosolly infected with virulent *M.tb*. and at week 6 post infection antibiotic treatment was initiated to reduce the bacterial load and establish a latent infection. During the latent stage the mice were vaccinated (as shown in figure 3) three times a 3 week intervals and the ability of the ESAT-6 vaccine to influence the number of polyfunctional T cells and to prevent reactivation of *M.tb* was determined 20 weeks after the last vaccination. The results showed that there was a substantial ESAT-6 response in the non-vaccinated group, but the cytokine expression profile was markedly different compared to the ESAT-6 vaccinated group (Fig. 5), in particularl in terms of polyfunctional T cells (IFN- $\gamma$  +TNF- $\alpha$ +IL-2+ CD4 T cells). Thus, compared to the non vaccinated group, we observed decreased numbers of IFN- $\gamma$ /TNF- $\alpha$  CD4 T cells, and increased numbers of triple positive polyfunctional CD4 T cells co-expressing IFN- $\gamma$ /TNF- $\alpha$ /IL-2. The increased presence of polyfunctional T cells correlated with decreased bacterial numbers in the lungs of ESAT-6 vaccinated animals (Fig. 5 D).

**EXAMPLE 6: Post-exposure vaccination with ESAT6 more consistently protects against reactivation compared to other antigens associated with both early and late stage**

## infection

**[0086]** To determine which antigens most consistently protect against reactivation we made a pooled analysis of normalized data based on all post-exposure experiments conducted. Data sets from individual experiments were normalized by comparing the bacterial load of each individual mouse within a group to the median of the control group, i.e. each data point represents the difference (Log10 CFU control median-Log10CFU vaccine group) between the control median CFU and the CFU of each individual animal (Figure 6). In figure 6A comparison of the pooled data set for protection for the latency associated antigens Rv0569, Rv2031c and the early antigens Ag85B, ESAT6, Rv3871, Rv3905 and Rv3445, of which the two latter are ESAT6 family proteins, shows that ESAT6 vaccinated animals are significantly better protected against reactivation compared to other antigens evaluated. Furthermore, protective levels attained following post-exposure vaccination with Rv3871, an ESX-1 protein, also seem to be elevated compared to the other antigens (Figure 6A). To further demonstrate the activity of ESAT6 in particular we compared the protection conferred by ESAT6 to the two fusion constructs H1 (Ag85B-ESAT6) and H56 (Ag85B-ESAT6-Rv2660) both of which contain ESAT6 (figure 6B). The analysis shows that ESAT6 activity still result in protection against reactivation when included in the two above mentioned fusion constructs.

**EXAMPLE 7: Postexposure vaccination with another member of the ESX-1 family, Rv3871, seems to have an inhibitory effect on the reactivation process**

**[0087]** We evaluated other members of the ESX-1 family in parallel with ESAT6 and found that Rv3871 postexposure vaccination led to an induction of Rv3871 specific immune response (Fig. 7B) although not to the extent of the ESAT6 induced immune response (Fig. 7A). Nevertheless both ESAT6 and Rv3871 induced immune responses were greater compared to saline control animals. The induction of vaccine specific immune response was associated with a lowered (median) bacterial load in both vaccine groups compared to the saline group. This indicated that Rv3871 may have a similar effect in protection against reactivation compared to ESAT6, demonstrated by the similar levels of bacterial numbers in these two groups compared to the somewhat elevated level in the control group (Figure 7C)

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## SEQUENCE LISTING

### [0089]

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					20			25					30		

Lys	Gln	Ser	Leu	Thr	Lys	Leu	Ala	Ala	Ala	Trp	Gly	Gly	Ser	Gly	Ser
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Glu	Ala	Tyr	Gln	Gly	Val	Gln	Gln	Lys	Trp	Asp	Ala	Thr	Ala	Thr	Glu
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Leu	Asn	Asn	Ala	Leu	Gln	Asn	Leu	Ala	Arg	Thr	Ile	Ser	Glu	Ala	Gly
					65			70		75			80		

Gln	Ala	Met	Ala	Ser	Thr	Glu	Gly	Asn	Val	Thr	Gly	Met	Phe	Ala	
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Asn	Phe	Glu	Arg	Ile	Ser	Gly	Asp	Leu	Lys	Thr	Gln	Ile	Asp	Gln	Val
				20				25		30					

Glu	Ser	Thr	Ala	Gly	Ser	Leu	Gln	Gly	Gln	Trp	Arg	Gly	Ala	Ala	Gly
				35			40		45						

Thr	Ala	Ala	Gln	Ala	Ala	Val	Val	Arg	Phe	Gln	Glu	Ala	Ala	Asn	Lys
					50			55		60					

Gln	Lys	Gln	Glu	Leu	Asp	Glu	Ile	Ser	Thr	Asn	Ile	Arg	Gln	Ala	Gly
				65			70		75		80				

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

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Asp Gly Arg Ile Asp His Val Glu Leu Ser Ala Arg Val Ala Trp Met  
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Arg Gln Lys Ala Gln Ser Ala Gln Tyr Ala Phe Ile Leu Asp Arg Met  
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Ser Gln Gln Val Asp Ala Asp Glu His Arg Val Ala Leu Leu Arg Lys  
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Thr Val Gly Glu Thr Trp Gly Leu Pro Ser Pro Glu Glu Ala Ala Ala  
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Ser Gln Phe Asn Asp Thr Leu Asn Val Tyr Leu Thr Ala His Asn Ala  
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 35 40 45

Arg Ser Gly Asn Arg Thr Asn Pro Ser Gly Ala Thr Met Ala Ala Leu  
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Gln Gly Asn Tyr Ala Asp Ala Met Glu Ala Leu Glu Ala Ala Pro Val  
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 145 150 155 160

Lys Trp Pro Asp Lys Phe Leu Ala Gly Ala Ala Gly Val Ala His Gly  
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Thr Glu Ala Asn Asp Ser Pro Ala Gly Glu Ala Cys Ala Arg Ala Ile  
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Val Ala Ala Leu Lys Asp Pro Ser Tyr Arg Leu Lys Thr Thr Thr  
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Val Thr Asp Asn Ser Gly Arg Glu Arg Leu Leu Ala Glu Ala Gln Ala  
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Glu Leu Asp Arg Gln Ile Gly Leu Thr Arg Val Lys Asn Gln Ile Glu  
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Arg Tyr Arg Ala Ala Thr Leu Met Ala Arg Val Arg Ala Ala Lys Gly  
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Met Lys Val Ala Gln Pro Ser Lys His Met Ile Phe Thr Gly Pro Pro  
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Gly Thr Gly Lys Thr Thr Ile Ala Arg Val Val Ala Asn Ile Leu Ala  
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Gly Leu Gly Val Ile Ala Glu Pro Lys Leu Val Glu Thr Ser Arg Lys  
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Thr Ile Asp Gln Ala Leu Gly Gly Val Leu Phe Ile Asp Glu Ala Tyr  
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Ser Ile Asp Pro Leu Gln Ser His Glu Ala Val Leu Val Ser Tyr Gln  
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Thr Asp Arg Ala Leu Thr Ser Ser Met Gly Ile Pro Val Thr Ala Arg  
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Asp Lys Gly Pro Gln Tyr Tyr Val Val Leu Pro Asp Gly Ile Ala Gln  
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Leu Val Ala Pro Pro Ala Met Val Pro Ser Leu Val Val Arg Ile Ala  
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Gly Asp Gln Ser Pro Gln Ser Thr Val Leu Ser Gly Arg His Leu Pro  
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Ile Ser Pro Ser Ala Met Asn Met Gly Ile Lys Gln Ile His Gly Thr  
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Arg Tyr Gly Val Pro Asn Ala Glu Thr Ala Lys Ser Leu Gly Leu Ser  
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Ser Pro Gln Asn Ala Pro Trp Glu Ile Val Arg Leu Leu Val Asp Gly  
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thr leu phe val val val asp glu phe ala glu leu leu gln ser his  
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Ala Gln Tyr Ile Thr Asn Lys Glu Ser Gly Val Gly Phe Leu Arg Val  
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Tyr Met Pro Pro Ala Ala Gly Val Glu Thr Asn Gly Glu Ala Gly Gly

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 35 40 45

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 65 70 75 80

Gly Ile Gly Gly Ala Pro Gln Thr Gly Lys Ser Thr Leu Leu Gln Thr  
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Phe Lys Glu His Arg Val Gly Ser Ile Gly Met Tyr Arg Gln Leu Arg  
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Glu Gly Gln Val Gln Asp Leu Ala Ala Gln Gly Leu Ala Phe Gly Val  
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His Val Ile Ile Ser Thr Pro Arg Trp Thr Glu Leu Lys Ser Arg Val  
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Arg Asp Tyr Leu Gly Thr Lys Ile Glu Phe Arg Leu Gly Asp Val Asn  
 245 250 255

Glu Thr Gln Ile Asp Arg Ile Thr Arg Glu Ile Pro Ala Asn Arg Pro  
 260 265 270

Gly Arg Ala Val Ser Met Glu Lys His His Leu Met Ile Gly Val Pro  
 275 280 285

Arg Phe Asp Gly Val His Ser Ala Asp Asn Leu Val Glu Ala Ile Thr  
 290 295 300

Ala Gly Val Thr Gln Ile Ala Ser Gln His Thr Glu Gln Ala Pro Pro  
 305 310 315 320

Val Arg Val Leu Pro Glu Arg Ile His Leu His Glu Leu Asp Pro Asn  
 325 330 335

Pro Pro Gly Pro Glu Ser Asp Tyr Arg Thr Arg Trp Glu Ile Pro Ile  
 340 345 350

Gly Leu Arg Glu Thr Asp Leu Thr Pro Ala His Cys His Met His Thr  
 355 360 365

Asn Pro His Leu Leu Ile Phe Gly Ala Ala Lys Ser Gly Lys Thr Thr  
 370 375 380

Ile Ala His Ala Ile Ala Arg Ala Ile Cys Ala Arg Asn Ser Pro Gln  
 385 390 395 400

Gln Val Arg Phe Met Leu Ala Asp Tyr Arg Ser Gly Leu Leu Asp Ala  
 405 410 415

Val Pro Asp Thr His Leu Leu Gly Ala Gly Ala Ile Asn Arg Asn Ser  
 420 425 430

Ala Ser Leu Asp Glu Ala Val Gln Ala Leu Ala Val Asn Leu Lys Lys  
 435 440 445

Arg Leu Pro Pro Thr Asp Leu Thr Thr Ala Gln Leu Arg Ser Arg Ser  
 450 455 460

Trp Trp Ser Gly Phe Asp Val Val Leu Val Asp Asp Trp His Met  
 465 470 475 480

Ile Val Gly Ala Ala Gly Gly Met Pro Pro Met Ala Pro Leu Ala Pro  
 485 490 495

Leu Leu Pro Ala Ala Ala Asp Ile Gly Leu His Ile Ile Val Thr Cys  
 500 505 510

Gln Met Ser Gln Ala Tyr Lys Ala Thr Met Asp Lys Phe Val Gly Ala  
 515 520 525

Ala Phe Gly Ser Gly Ala Pro Thr Met Phe Leu Ser Gly Glu Lys Gln  
 530 535 540

Glu Phe Pro Ser Ser Glu Phe Lys Val Lys Arg Arg Pro Pro Gly Gln  
 545 550 555 560

Ala Phe Leu Val Ser Pro Asp Gly Lys Glu Val Ile Gln Ala Pro Tyr  
 565 570 575

Ile Glu Pro Pro Glu Glu Val Phe Ala Ala Pro Pro Ser Ala Gly  
 580 585 590

<210> 11

<211> 99

<212> PRT

<213> Mycobacterium tuberculosis

<400> 11

Met Glu Lys Met Ser His Asp Pro Ile Ala Ala Asp Ile Gly Thr Gln  
 1 5 10 15

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

Thr Ser Val Thr Gly Leu Val Pro Ala Gly Ala Asp Glu Val Ser Ala  
 35 40 45

Gln Ala Ala Thr Ala Phe Thr Ser Glu Gly Ile Gln Leu Leu Ala Ser  
 50 55 60

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

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

Phe Ala Glu.

<210> 12

<211> 368

<212> PRT

<213> Mycobacterium tuberculosis

<400> 12

Met Leu Trp His Ala Met Pro Pro Glu Leu Asn Thr Ala Arg Leu Met  
 1 5 10 15

Ala Gly Ala Gly Pro Ala Pro Met Leu Ala Ala Ala Ala Gly Trp Gln  
 20 25 30

Thr Leu Ser Ala Ala Leu Asp Ala Gln Ala Val Glu Leu Thr Ala Arg  
 35 40 45

Leu Asn Ser Leu Gly Glu Ala Trp Thr Gly Gly Ser Asp Lys Ala  
 50 55 60

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

Gln Ala Lys Thr Arg Ala Met Gln Ala Thr Ala Gln Ala Ala Ala Tyr  
 85 90 95

Thr Gln Ala Met Ala Thr Thr Pro Ser Leu Pro Glu Ile Ala Ala Asn  
 100 105 110

His Ile Thr Gln Ala Val Leu Thr Ala Thr Asn Phe Phe Gly Ile Asn  
 115 120 125

Thr Ile Pro Ile Ala Leu Thr Glu Met Asp Tyr Phe Ile Arg Met Trp  
 130 135 140

Asn Gln Ala Ala Leu Ala Met Glu Val Tyr Gln Ala Glu Thr Ala Val  
 145 150 155 160

Asn Thr Leu Phe Glu Lys Leu Glu Pro Met Ala Ser Ile Leu Asp Pro  
 165 170 175

Gly Ala Ser Gln Ser Thr Thr Asn Pro Ile Phe Gly Met Pro Ser Pro  
 180 185 190

Gly Ser Ser Thr Pro Val Gly Gln Leu Pro Pro Ala Ala Thr Gln Thr  
 195 200 205

Leu Gly Gln Leu Gly Glu Met Ser Gly Pro Met Gln Gln Leu Thr Gln  
 210 215 220

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

Gly Gly Asn Pro Ala Asp Glu Glu Ala Ala Gln Met Gly Leu Leu Gly  
 245 250 255

Thr Ser Pro Leu Ser Asn His Pro Leu Ala Gly Gly Ser Gly Pro Ser  
 260 265 270

Ala Gly Ala Gly Leu Leu Arg Ala Glu Ser Leu Pro Gly Ala Gly Gly  
 275 280 285

Ser Leu Thr Arg Thr Pro Leu Met Ser Gln Leu Ile Glu Lys Pro Val  
 290 295 300

Ala Pro Ser Val Met Pro Ala Ala Ala Gly Ser Ser Ala Thr Gly  
 305 310 315 320

Gly Ala Ala Pro Val Gly Ala Gly Ala Met Gly Gln Gly Ala Gln Ser  
 325 330 335

Gly Gly Ser Thr Arg Pro Gly Leu Val Ala Pro Ala Pro Leu Ala Gln  
 340 345 350

Glu Arg Glu Glu Asp Asp Glu Asp Asp Trp Asp Glu Glu Asp Asp Trp  
 355 360 365

<210> 13

<211> 666

<212> PRT

<213> Mycobacterium tuberculosis

<400> 13

Met Ala Ala Asp Tyr Asp Lys Leu Phe Arg Pro His Glu Gly Met Glu  
 1 5 10 15

Ala Pro Asp Asp Met Ala Ala Gln Pro Phe Phe Asp Pro Ser Ala Ser  
 20 25 30

Phe Pro Pro Ala Pro Ala Ser Ala Asn Leu Pro Lys Pro Asn Gly Gln  
 35 40 45

Thr Pro Pro Pro Thr Ser Asp Asp Leu Ser Glu Arg Phe Val Ser Ala  
 50 55 60

Pro Thr Pro Met  
 65 70 75 80

Pro Ile Ala Ala Gly Glu Pro Pro Ser Pro Glu Pro Ala Ala Ser Lys  
 85 90 95

Pro Pro Thr Pro Pro Met Pro Ile Ala Gly Pro Glu Pro Ala Pro Pro  
 100 105 110

Lys Pro Pro Thr Pro Pro Met Pro Ile Ala Gly Pro Glu Pro Ala Pro  
 115 120 125

Pro Lys Pro Pro Thr Pro Pro Met Pro Ile Ala Gly Pro Ala Pro Thr

130 135 140

Pro Thr Glu Ser Gln Leu Ala Pro Pro Arg Pro Pro Thr Pro Gln Thr  
145 150 155 160

Pro Thr Gly Ala Pro Gln Gln Pro Glu Ser Pro Ala Pro His Val Pro  
165 170 175

Ser His Gly Pro His Gln Pro Arg Arg Thr Ala Pro Ala Pro Pro Trp  
180 185 190

Ala Lys Met Pro Ile Gly Glu Pro Pro Pro Ala Pro Ser Arg Pro Ser  
195 200 205

Ala Ser Pro Ala Glu Pro Pro Thr Arg Pro Ala Pro Gln His Ser Arg  
210 215 220

Arg Ala Arg Arg Gly His Arg Tyr Arg Thr Asp Thr Glu Arg Asn Val  
225 230 235 240

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

Glu Ala Ser Gly Ala Gln Leu Ala Pro Gly Thr Glu Pro Ser Pro Ala  
260 265 270

Pro Leu Gly Gln Pro Arg Ser Tyr Leu Ala Pro Pro Thr Arg Pro Ala  
275 280 285

Pro Thr Glu Pro Pro Pro Ser Pro Ser Pro Gln Arg Asn Ser Gly Arg  
290 295 300

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

Ala Gln Pro Asp Ser Ile Thr Ala Ala Thr Thr Gly Gly Arg Arg Arg  
325 330 335

Lys Arg Ala Ala Pro Asp Leu Asp Ala Thr Gln Lys Ser Leu Arg Pro  
340 345 350

Ala Ala Lys Gly Pro Lys Val Lys Lys Val Lys Pro Gln Lys Pro Lys  
355 360 365

Ala Thr Lys Pro Pro Lys Val Val Ser Gln Arg Gly Trp Arg His Trp  
370 375 380

Val His Ala Leu Thr Arg Ile Asn Leu Gly Leu Ser Pro Asp Glu Lys  
385 390 395 400

Tyr Glu Leu Asp Leu His Ala Arg Val Arg Arg Asn Pro Arg Gly Ser  
405 410 415

Tyr Gln Ile Ala Val Val Gly Leu Lys Gly Gly Ala Gly Lys Thr Thr  
420 425 430

Leu Thr Ala Ala Leu Gly Ser Thr Leu Ala Gln Val Arg Ala Asp Arg

435	440	445
Ile Leu Ala Leu Asp Ala Asp Pro Gly Ala Gly Asn Leu Ala Asp Arg		
450	455	460
Val Gly Arg Gln Ser Gly Ala Thr Ile Ala Asp Val Leu Ala Glu Lys		
465	470	475
480		
Glu Leu Ser His Tyr Asn Asp Ile Arg Ala His Thr Ser Val Asn Ala		
485	490	495
Val Asn Leu Glu Val Leu Pro Ala Pro Glu Tyr Ser Ser Ala Gln Arg		
500	505	510
Ala Leu Ser Asp Ala Asp Trp His Phe Ile Ala Asp Pro Ala Ser Arg		
515	520	525
Phe Tyr Asn Leu Val Leu Ala Asp Cys Gly Ala Gly Phe Phe Asp Pro		
530	535	540
Leu Thr Arg Gly Val Leu Ser Thr Val Ser Gly Val Val Val Val Ala		
545	550	555
560		
Ser Val Ser Ile Asp Gly Ala Gln Gln Ala Ser Val Ala Leu Asp Trp		
565	570	575
Leu Arg Asn Asn Gly Tyr Gln Asp Leu Ala Ser Arg Ala Cys Val Val		
580	585	590
Ile Asn His Ile Met Pro Gly Glu Pro Asn Val Ala Val Lys Asp Leu		
595	600	605
Val Arg His Phe Glu Gln Gln Val Gln Pro Gly Arg Val Val Val Met		
610	615	620
Pro Trp Asp Arg His Ile Ala Ala Gly Thr Glu Ile Ser Leu Asp Leu		
625	630	635
640		
Leu Asp Pro Ile Tyr Lys Arg Lys Val Leu Glu Leu Ala Ala Ala Leu		
645	650	655
Ser Asp Asp Phe Glu Arg Ala Gly Arg Arg		
660	665	
<210> 14		
<211> 511		
<212> PRT		
<213> Mycobacterium tuberculosis		
<400> 14		
Leu Ser Ala Pro Ala Val Ala Ala Gly Pro Thr Ala Ala Gly Ala Thr		
1	5	10
15		
Ala Ala Arg Pro Ala Thr Thr Arg Val Thr Ile Leu Thr Gly Arg Arg		
20	25	30

Met Thr Asp Leu Val Leu Pro Ala Ala Val Pro Met Glu Thr Tyr Ile  
 35 40 45

Asp Asp Thr Val Ala Val Ser Glu Val Leu Glu Asp Thr Pro Ala  
 50 55 60

Asp Val Leu Gly Gly Phe Asp Phe Thr Ala Gln Gly Val Trp Ala Phe  
 65 70 75 80

Ala Arg Pro Gly Ser Pro Pro Leu Lys Leu Asp Gln Ser Leu Asp Asp  
 85 90 95

Ala Gly Val Val Asp Gly Ser Leu Leu Thr Leu Val Ser Val Ser Arg  
 100 105 110

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

Val Leu Asp Glu Ser Pro Glu Phe Asp Arg Thr Ala Leu Asn Arg Phe  
 130 135 140

Val Gly Ala Ala Ile Pro Leu Leu Thr Ala Pro Val Ile Gly Met Ala  
 145 150 155 160

Met Arg Ala Trp Trp Glu Thr Gly Arg Ser Leu Trp Trp Pro Leu Ala  
 165 170 175

Ile Gly Ile Leu Gly Ile Ala Val Leu Val Gly Ser Phe Val Ala Asn  
 180 185 190

Arg Phe Tyr Gln Ser Gly His Leu Ala Glu Cys Leu Leu Val Thr Thr  
 195 200 205

Tyr Leu Leu Ile Ala Thr Ala Ala Leu Ala Val Pro Leu Pro Arg  
 210 215 220

Gly Val Asn Ser Leu Gly Ala Pro Gln Val Ala Gly Ala Ala Thr Ala  
 225 230 235 240

Val Leu Phe Leu Thr Leu Met Thr Arg Gly Gly Pro Arg Lys Arg His  
 245 250 255

Glu Leu Ala Ser Phe Ala Val Ile Thr Ala Ile Ala Val Ile Ala Ala  
 260 265 270

Ala Ala Ala Phe Gly Tyr Gly Tyr Gln Asp Trp Val Pro Ala Gly Gly  
 275 280 285

Ile Ala Phe Gly Leu Phe Ile Val Thr Asn Ala Ala Lys Leu Thr Val  
 290 295 300

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

Val Asp Asn Glu Glu Leu Leu Asp Pro Val Ala Thr Pro Glu Ala Thr  
 325 330 335

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

Ser Ala Val Arg Leu Thr Glu Arg Ser Lys Leu Ala Lys Gln Leu Leu  
 355 360 365

Ile Gly Tyr Val Thr Ser Gly Thr Leu Ile Leu Ala Ala Gly Ala Ile  
 370 375 380

Ala Val Val Val Arg Gly His Phe Phe Val His Ser Leu Val Val Ala  
 385 390 395 400

Gly Leu Ile Thr Thr Val Cys Gly Phe Arg Ser Arg Leu Tyr Ala Glu  
 405 410 415

Arg Trp Cys Ala Trp Ala Leu Leu Ala Ala Thr Val Ala Ile Pro Thr  
 420 425 430

Gly Leu Thr Ala Lys Leu Ile Ile Trp Tyr Pro His Tyr Ala Trp Leu  
 435 440 445

Leu Leu Ser Val Tyr Leu Thr Val Ala Leu Val Ala Leu Val Val Val  
 450 455 460

Gly Ser Met Ala His Val Arg Arg Val Ser Pro Val Val Lys Arg Thr  
 465 470 475 480

Leu Glu Leu Ile Asp Gly Ala Met Ile Ala Ala Ile Ile Pro Met Leu  
 485 490 495

Leu Trp Ile Thr Gly Val Tyr Asp Thr Val Arg Asn Ile Arg Phe  
 500 505 510

<210> 15

<211> 280

<212> PRT

<213> Mycobacterium tuberculosis

<400> 15

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

Ala Lys Leu Ala Gly Leu Val Phe Pro Gln Pro Pro Ala Pro Ile Ala  
 20 25 30

Val Ser Gly Thr Asp Ser Val Val Ala Ala Ile Asn Glu Thr Met Pro  
 35 40 45

Ser Ile Glu Ser Leu Val Ser Asp Gly Leu Pro Gly Val Lys Ala Ala  
 50 55 60

Leu Thr Arg Thr Ala Ser Asn Met Asn Ala Ala Ala Asp Val Tyr Ala  
 65 70 75 80

Lys Thr Asp Gln Ser Leu Gly Thr Ser Leu Ser Gln Tyr Ala Phe Gly  
 75 80 85

85

90

95

Ser Ser Gly Glu Gly Leu Ala Gly Val Ala Ser Val Gly Gly Gln Pro  
 100 105 110

Ser Gln Ala Thr Gln Leu Leu Ser Thr Pro Val Ser Gln Val Thr Thr  
 115 120 125

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

Val Pro Gln Leu Val Gln Leu Ala Pro His Ala Val Gln Met Ser Gln  
 145 150 155 160

Asn Ala Ser Pro Ile Ala Gln Thr Ile Ser Gln Thr Ala Gln Gln Ala  
 165 170 175

Ala Gln Ser Ala Gln Gly Gly Ser Gly Pro Met Pro Ala Gln Leu Ala  
 180 185 190

Ser Ala Glu Lys Pro Ala Thr Glu Gln Ala Glu Pro Val His Glu Val  
 195 200 205

Thr Asn Asp Asp Gln Gly Asp Gln Gly Asp Val Gln Pro Ala Glu Val  
 210 215 220

Val Ala Ala Ala Arg Asp Glu Gly Ala Gly Ala Ser Pro Gly Gln Gln  
 225 230 235 240

Pro Gly Gly Gly Val Pro Ala Gln Ala Met Asp Thr Gly Ala Gly Ala  
 245 250 255

Arg Pro Ala Ala Ser Pro Leu Ala Ala Pro Val Asp Pro Ser Thr Pro  
 260 265 270

Ala Pro Ser Thr Thr Thr Thr Leu  
 275 280

<210> 16

<211> 729

<212> PRT

<213> Mycobacterium tuberculosis

<400> 16

Met Ser Ile Thr Arg Pro Thr Gly Ser Tyr Ala Arg Gln Met Leu Asp  
 1 5 10 15

Pro Gly Gly Trp Val Glu Ala Asp Glu Asp Thr Phe Tyr Asp Arg Ala  
 20 25 30

Gln Glu Tyr Ser Gln Val Leu Gln Arg Val Thr Asp Val Leu Asp Thr  
 35 40 45

Cys Arg Gln Gln Lys Glv His Val Phe Glu Glv Glv Leu Trp Ser Glv

50	55	60
Gly Ala Ala Asn Ala Ala Asn Gly Ala Leu Gly Ala Asn Ile Asn Gln		
65	70	75
Leu Met Thr Leu Gln Asp Tyr Leu Ala Thr Val Ile Thr Trp His Arg		
85	90	95
His Ile Ala Gly Leu Ile Glu Gln Ala Lys Ser Asp Ile Gly Asn Asn		
100	105	110
Val Asp Gly Ala Gln Arg Glu Ile Asp Ile Leu Glu Asn Asp Pro Ser		
115	120	125
Leu Asp Ala Asp Glu Arg His Thr Ala Ile Asn Ser Leu Val Thr Ala		
130	135	140
Thr His Gly Ala Asn Val Ser Leu Val Ala Glu Thr Ala Glu Arg Val		
145	150	155
Leu Glu Ser Lys Asn Trp Lys Pro Pro Lys Asn Ala Leu Glu Asp Leu		
165	170	175
Leu Gln Gln Lys Ser Pro Pro Pro Asp Val Pro Thr Leu Val Val		
180	185	190
Pro Ser Pro Gly Thr Pro Gly Thr Pro Gly Thr Pro Ile Thr Pro Gly		
195	200	205
Thr Pro Ile Thr Pro Gly Thr Pro Ile Thr Pro Ile Pro Gly Ala Pro		
210	215	220
Val Thr Pro Ile Thr Pro Thr Pro Gly Thr Pro Val Thr Pro Val Thr		
225	230	235
240		
Pro Gly Lys Pro Val Thr Pro Val Thr Pro Val Lys Pro Gly Thr Pro		
245	250	255
Gly Glu Pro Thr Pro Ile Thr Pro Val Thr Pro Pro Val Ala Pro Ala		
260	265	270
Thr Pro Ala Thr Pro Ala Thr Pro Val Thr Pro Ala Pro Ala Pro His		
275	280	285
Pro Gln Pro Ala Pro Ala Pro Ala Pro Ser Pro Gly Pro Gln Pro Val		
290	295	300
Thr Pro Ala Thr Pro Gly Pro Ser Gly Pro Ala Thr Pro Gly Thr Pro		
305	310	315
320		
Gly Gly Glu Pro Ala Pro His Val Lys Pro Ala Ala Leu Ala Glu Gln		
325	330	335
Pro Gly Val Pro Gly Gln His Ala Gly Gly Thr Gln Ser Gly Pro		
340	345	350
Ala His Ala Asp Glu Ser Ala Ala Ser Val Thr Pro Ala Ala Ala Ser		
355	360	365

Gly Val Pro Gly Ala Arg Ala Ala Ala Ala Pro Ser Gly Thr Ala  
 370 375 380

Val Gly Ala Gly Ala Arg Ser Ser Val Gly Thr Ala Ala Ala Ser Gly  
 385 390 395 400

Ala Gly Ser His Ala Ala Thr Gly Arg Ala Pro Val Ala Thr Ser Asp  
 405 410 415

Lys Ala Ala Ala Pro Ser Thr Arg Ala Ala Ser Ala Arg Thr Ala Pro  
 420 425 430

Pro Ala Arg Pro Pro Ser Thr Asp His Ile Asp Lys Pro Asp Arg Ser  
 435 440 445

Glu Ser Ala Asp Asp Gly Thr Pro Val Ser Met Ile Pro Val Ser Ala  
 450 455 460

Ala Arg Ala Ala Arg Asp Ala Ala Thr Ala Ala Ala Ser Ala Arg Gln  
 465 470 475 480

Arg Gly Arg Gly Asp Ala Leu Arg Leu Ala Arg Arg Ile Ala Ala Ala  
 485 490 495

Leu Asn Ala Ser Asp Asn Asn Ala Gly Asp Tyr Gly Phe Phe Trp Ile  
 500 505 510

Thr Ala Val Thr Thr Asp Gly Ser Ile Val Val Ala Asn Ser Tyr Gly  
 515 520 525

Leu Ala Tyr Ile Pro Asp Gly Met Glu Leu Pro Asn Lys Val Tyr Leu  
 530 535 540

Ala Ser Ala Asp His Ala Ile Pro Val Asp Glu Ile Ala Arg Cys Ala  
 545 550 555 560

Thr Tyr Pro Val Leu Ala Val Gln Ala Trp Ala Ala Phe His Asp Met  
 565 570 575

Thr Leu Arg Ala Val Ile Gly Thr Ala Glu Gln Leu Ala Ser Ser Asp  
 580 585 590

Pro Gly Val Ala Lys Ile Val Leu Glu Pro Asp Asp Ile Pro Glu Ser

595 600 605

Gly Lys Met Thr Gly Arg Ser Arg Leu Glu Val Val Asp Pro Ser Ala  
 610 615 620

Ala Ala Gln Leu Ala Asp Thr Thr Asp Gln Arg Leu Leu Asp Leu Leu  
 625 630 635 640

Pro Pro Ala Pro Val Asp Val Asn Pro Pro Gly Asp Glu Arg His Met  
 645 650 655

Leu Trp Phe Glu Leu Met Lys Pro Met Thr Ser Thr Ala Thr Gly Arg  
 660 665 670

Glu Ala Ala His Leu Arg Ala Phe Arg Ala Tyr Ala Ala His Ser Gln  
 675 680 685

Glu Ile Ala Leu His Gln Ala His Thr Ala Thr Asp Ala Ala Val Gln  
 690 695 700

Arg Val Ala Val Ala Asp Trp Leu Tyr Trp Gln Tyr Val Thr Gly Leu  
 705 710 715 720

Leu Asp Arg Ala Leu Ala Ala Ala Cys  
 725

<210> 17

<211> 115

<212> PRT

<213> Mycobacterium tuberculosis

<400> 17

Val Ser Met Asp Glu Leu Asp Pro His Val Ala Arg Ala Leu Thr Leu  
 1 5 10 15

Ala Ala Arg Phe Gln Ser Ala Leu Asp Gly Thr Leu Asn Gln Met Asn  
 20 25 30

Asn Gly Ser Phe Arg Ala Thr Asp Glu Ala Glu Thr Val Glu Val Thr  
 35 40 45

Ile Asn Gly His Gln Trp Leu Thr Gly Leu Arg Ile Glu Asp Gly Leu  
 50 55 60

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

Leu His Asn Ala Gln Ala Ala Ser Ala Tyr Asn Asp Ala Ala Gly  
 85 90 95

Glu Gln Leu Thr Ala Ala Leu Ser Ala Met Ser Arg Ala Met Asn Glu  
 100 105 110

Gly Met Ala  
 115

<210> 18

<211> 460

<212> PRT

<213> Mycobacterium tuberculosis

<400> 18

Met Thr Gln Ser Gln Thr Val Thr Val Asp Gln Gln Glu Ile Leu Asn  
 1 5 10 15

Arg Ala Asn Glu Val Glu Ala Pro Met Ala Asp Pro Pro Thr Asp Val  
 20 25 30

Pro Ile Thr Pro Cys Glu Leu Thr Ala Ala Lys Asn Ala Ala Gln Gln  
 35 40 45

Leu Val Leu Ser Ala Asp Asn Met Arg Glu Tyr Leu Ala Ala Gly Ala  
 50 55 60

Lys Glu Arg Gln Arg Leu Ala Thr Ser Leu Arg Asn Ala Ala Lys Ala  
 65 70 75 80

Tyr Gly Glu Val Asp Glu Glu Ala Ala Thr Ala Leu Asp Asn Asp Gly  
 85 90 95

Glu Gly Thr Val Gln Ala Glu Ser Ala Gly Ala Val Gly Gly Asp Ser  
 100 105 110

Ser Ala Glu Leu Thr Asp Thr Pro Arg Val Ala Thr Ala Gly Glu Pro  
 115 120 125

Asn Phe Met Asp Leu Lys Glu Ala Ala Arg Lys Leu Glu Thr Gly Asp  
 130 135 140

Gln Gly Ala Ser Leu Ala His Phe Ala Asp Gly Trp Asn Thr Phe Asn  
 145 150 155 160

Leu Thr Leu Gln Gly Asp Val Lys Arg Phe Arg Gly Phe Asp Asn Trp  
 165 170 175

Glu Gly Asp Ala Ala Thr Ala Cys Glu Ala Ser Leu Asp Gln Gln Arg  
 180 185 190

Gln Trp Ile Leu His Met Ala Lys Leu Ser Ala Ala Met Ala Lys Gln  
 195 200 205

Ala Gln Tyr Val Ala Gln Leu His Val Trp Ala Arg Arg Glu His Pro  
 210 215 220

Thr Tyr Glu Asp Ile Val Gly Leu Glu Arg Leu Tyr Ala Glu Asn Pro  
 225 230 235 240

Ser Ala Arg Asp Gln Ile Leu Pro Val Tyr Ala Glu Tyr Gln Gln Arg  
 245 250 255

Ser Glu Lys Val Leu Thr Glu Tyr Asn Asn Lys Ala Ala Leu Glu Pro  
 260 265 270

Val Asn Pro Pro Lys Pro Pro Ala Ile Lys Ile Asp Pro Pro Pro  
 275 280 285

Pro Pro Gln Glu Gln Gly Leu Ile Pro Gly Phe Leu Met Pro Pro Ser  
 290 295 300

Asp Gly Ser Gly Val Thr Pro Gly Thr Gly Met Pro Ala Ala Pro Met  
 305 310 315 320

Val Pro Pro Thr Gly Ser Pro Gly Gly Leu Pro Ala Asp Thr Ala  
 325 330 335

Ala Gln Leu Thr Ser Ala Gly Arg Glu Ala Ala Ala Leu Ser Gly Asp  
340 345 350

Val Ala Val Lys Ala Ala Ser Leu Gly Gly Gly Gly Gly Gly Val  
355 360 365

Pro Ser Ala Pro Leu Gly Ser Ala Ile Gly Gly Ala Glu Ser Val Arg  
370 375 380

Pro Ala Gly Ala Gly Asp Ile Ala Gly Leu Gly Gln Gly Arg Ala Gly  
385 390 395 400

Gly Gly Ala Ala Leu Gly Gly Gly Met Gly Met Pro Met Gly Ala  
405 410 415

Ala His Gln Gly Gln Gly Ala Lys Ser Lys Gly Ser Gln Gln Glu  
420 425 430

Asp Glu Ala Leu Tyr Thr Glu Asp Arg Ala Trp Thr Glu Ala Val Ile  
435 440 445

Gly Asn Arg Arg Arg Gln Asp Ser Lys Glu Ser Lys  
450 455 460

<210> 19

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 19

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

<210> 20

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 20

Asn Phe Ala Gly Ile Glu Ala Ala Ala Ser Ala Ile Gln Gly Asn  
1 5 10 15

<210> 21

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 21

Ala Ser Ala Ile Gln Gly Asn Val Thr Ser Ile His Ser Leu Leu  
1 5 10 15

<210> 22

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 22

Asn Val Thr Ser Ile His Ser Leu Leu Asp Glu Gly Lys Gln Ser  
1 5 10 15

<210> 23

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 23

Ser Leu Leu Asp Glu Gly Lys Gln Ser Leu Thr Lys Leu Ala Ala  
1 5 10 15

<210> 24

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 24

Lys Gln Ser Leu Thr Lys Leu Ala Ala Ala Trp Gly Gly Ser Gly

1 5 10 15

<210> 25

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 25

Ala Ala Trp Gly Gly Ser Gly Ser Glu Ala Tyr Gln Gly Val Gln  
1 5 10 15

<210> 26

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 26

Gly Ser Glu Ala Tyr Gln Gly Val Gln Gln Lys Trp Asp Ala Thr  
1 5 10 15

<210> 27

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 27

Gln Gln Lys Trp Asp Ala Thr Ala Thr Glu Leu Asn Asn Ala Leu  
1 5 10 15

<210> 28

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 28

Thr Ala Thr Glu Leu Asn Asn Ala Leu Gln Asn Leu Ala Arg Thr  
1 5 10 15

<210> 29

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 29

Ala Leu Gln Asn Leu Ala Arg Thr Ile Ser Glu Ala Gly Gln Ala  
1 5 10 15

<210> 30

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 30

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

<210> 31

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 31

Gln Ala Met Ala Ser Thr Glu Gly Asn Val Thr Gly Met Phe Ala  
1 5 10 15

<210> 32

<211> 462

<212> PRT

<213> Mycobacterium tuberculosis

<400> 32

Met Arg Asn Pro Leu Gly Leu Arg Phe Ser Thr Gly His Ala Leu Leu  
1 5 10 15

Ala Ser Ala Leu Ala Pro Pro Cys Ile Ile Ala Phe Leu Glu Thr Arg  
20 25 30

Tyr Trp Trp Ala Gly Ile Ala Leu Ala Ser Leu Gly Val Ile Val Ala  
35 40 45

Thr Val Thr Phe Tyr Gly Arg Arg Ile Thr Gly Trp Val Ala Ala Val  
50 55 60

Tyr Ala Trp Leu Arg Arg Arg Arg Arg Pro Pro Asp Ser Ser Ser Glu  
 65 70 75 80

Pro Val Val Gly Ala Thr Val Lys Pro Gly Asp His Val Ala Val Arg  
 85 90 95

Trp Gln Gly Glu Phe Leu Val Ala Val Ile Glu Leu Ile Pro Arg Pro  
 100 105 110

Phe Thr Pro Thr Val Ile Val Asp Gly Gln Ala His Thr Asp Asp Met  
 115 120 125

Leu Asp Thr Gly Leu Val Glu Glu Leu Leu Ser Val His Cys Pro Asp  
 130 135 140

Leu Glu Ala Asp Ile Val Ser Ala Gly Tyr Arg Val Gly Asn Thr Ala  
 145 150 155 160

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

Ala Pro Ala Asn Arg Arg Thr Trp Ile Val Leu Arg Ala Asp Pro Glu  
 180 185 190

Arg Thr Arg Lys Ser Ala Gln Arg Arg Asp Glu Gly Val Ala Gly Leu  
 195 200 205

Ala Arg Tyr Leu Val Ala Ser Ala Thr Arg Ile Ala Asp Arg Leu Ala  
 210 215 220

Ser His Gly Val Asp Ala Val Cys Gly Arg Ser Phe Asp Asp Tyr Asp  
 225 230 235 240

His Ala Thr Asp Ile Gly Phe Val Arg Glu Lys Trp Ser Met Ile Lys  
 245 250 255

Gly Arg Asp Ala Tyr Thr Ala Ala Tyr Ala Ala Pro Gly Gly Pro Asp  
 260 265 270

Val Trp Trp Ser Ala Arg Ala Asp His Thr Ile Thr Arg Val Arg Val  
 275 280 285

Ala Pro Gly Met Ala Pro Gln Ser Thr Val Leu Leu Thr Thr Ala Asp  
 290 295 300

Lys Pro Lys Thr Pro Arg Gly Phe Ala Arg Leu Phe Gly Gly Gln Arg  
 305 310 315 320

Pro Ala Leu Gln Gly Gln His Leu Val Ala Asn Arg His Cys Gln Leu  
 325 330 335

Pro Ile Gly Ser Ala Gly Val Leu Val Gly Glu Thr Val Asn Arg Cys  
 340 345 350

Pro Val Tyr Met Pro Phe Asp Asp Val Asp Ile Ala Leu Asn Leu Gly  
 355 360 365

Asp Ala Gln Thr Phe Thr Gln Phe Val Val Arg Ala Ala Ala Ala Gly  
 370 375 380

Ala Met Val Thr Val Gly Pro Gln Phe Glu Glu Phe Ala Arg Leu Ile  
 385 390 395 400

Gly Ala His Ile Gly Gln Glu Val Lys Val Ala Trp Pro Asn Ala Thr  
 405 410 415

Thr Tyr Leu Gly Pro His Pro Gly Ile Asp Arg Val Ile Leu Arg His  
 420 425 430

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Pro Pro Asp Val Thr Gly Pro Asp Gln Pro Thr Glu Gln Arg Val Leu  
 35 40 45

Cys Ala Ser Pro Thr Thr Leu Pro Gly Ser Gly Phe His Asp Pro Pro  
 50 55 60

Trp Ser Asn Thr Tyr Leu Gly Val Ala Asp Ala His Lys Phe Ala Thr  
 65 70 75 80

Gly Ala Gly Val Thr Val Ala Val Ile Asp Thr Gly Val Asp Ala Ser  
 85 90 95

Pro Arg Val Pro Ala Glu Pro Gly Gly Asp Phe Val Asp Gln Ala Gly  
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Asn Gly Leu Ser Asp Cys Asp Ala His Gly Thr Leu Thr Ala Ser Ile  
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Ile Ala Gly Arg Pro Ala Pro Thr Asp Gly Phe Val Gly Val Ala Pro  
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Asp Ala Arg Leu Leu Ser Leu Arg Gln Thr Ser Glu Ala Phe Glu Pro  
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Val Gly Ser Gln Ala Asn Pro Asn Asp Pro Asn Ala Thr Pro Ala Ala

165

170

175

Gly Ser Ile Arg Ser Leu Ala Arg Ala Val Val His Ala Ala Asn Leu  
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Gly Val Gly Val Ile Asn Ile Ser Glu Ala Ala Cys Tyr Lys Val Ser  
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Arg Pro Ile Asp Glu Thr Ser Leu Gly Ala Ser Ile Asp Tyr Ala Val  
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Asn Val Lys Gly Val Val Val Val Ala Ala Gly Asn Thr Gly Gly  
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Asp Cys Val Gln Asn Pro Ala Pro Asp Pro Ser Thr Pro Gly Asp Pro  
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Arg Gly Trp Asn Asn Val Gln Thr Val Val Thr Pro Ala Trp Tyr Ala  
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Ser Phe Ser Met His Gly Pro Trp Val Asp Val Ala Ala Pro Ala Glu  
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Tyr Val Ser Gly Leu Ala Ala Leu Leu Arg Gln Arg Phe Pro Asp Leu  
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Thr Pro Ala Gln Ile Ile His Arg Ile Thr Ala Thr Ala Arg His Pro  
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Ala Ala Leu Thr Trp Asp Ile Pro Pro Gly Pro Ala Ser Ala Pro Tyr  
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Asn Val Arg Arg Leu Pro Pro Val Val Glu Pro Gly Pro Asp Arg  
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35 40 45

Ser Lys Phe Asn Asp Thr Leu Gln Glu Phe Glu Thr Thr Arg Ser Ser  
50 55 60

Thr Gly Thr Gly Leu Gln Gly Val Thr Ser Gly Leu Ala Asn Asn Leu  
65 70 75 80

Leu Ala Ala Ala Gly Ala Tyr Leu Lys Ala Asp Asp Gly Leu Ala Gly  
85 90 95

Val Ile Asp Lys Ile Phe Gly  
100

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**Patentkrav**

- 1.** Vaccine til anvendelse til forebyggelse af reaktivering af tuberkulose hos individer, der er latent inficeret med M.tuberkulose, omfattende et antigen der 5 udtrykkes konstitutivt under infektion med M.tuberkulose eller en nukleinsyre der koder for antigenet, hvor antigenet, der tilhører ESX-1-sekretionssystemet, er valgt fra gruppen bestående af
  - i) ESAT6, CFP10, Rv3614c, Rv3615c, EspR, Rv3868, Rv3869, Rv3870, 10 Rv3871, Rv3872, Rv3873, Rv3876, Rv3877, Rv3878, Rv3879c, Rv3880c, Rv3881c, Rv3882c, Rv3883c og Rv3865,
  - ii) en immunogen del omfattende en epitop for en B-celle eller T-celle af en hvilken som helst af sekvenserne i (i); og
  - iii) en aminosyresekvvensanalog med mindst 90% sekvensidentitet med en 15 hvilken som helst af sekvenserne i (i) eller (ii), og som samtidig er immunogen, idet den forebygger reaktivering af latent tuberkuloseinfektion, når den indgives som en terapeutisk vaccine.
- 2.** Vaccine til anvendelse ifølge krav 1, hvor polypeptiderne er fusioneret til et 20 antigen udtrykt af bakterier fra mykobakterie-familien.
- 3.** Vaccine til anvendelse ifølge krav 2, hvor fusionspartneren er et antigen, der er konstitutivt udtrykt.
- 25 **4.** Vaccine til anvendelse ifølge krav 3, omfattende ESAT6 fusioneret til CFP10.
- 5.** Vaccine til anvendelse ifølge et hvilket som helst af de foregående krav, omfattende et yderligere indgivelsessystem valgt blandt, levende rekombinante vacciner, der er gen-modificerede organismer såsom bakterier eller vira der 30 udtrykker mykobakterielle gener, eller immunogene indgivelsessystemer såsom, DNA-vacciner, der er plasmider der udtrykker gener eller genfragmenter for proteinerne beskrevet ovenfor, eller proteinvacciner, der er proteinerne selv eller syntetiske peptider afledt fra proteinerne selv indgivet i et indgivelsessystem

såsom en adjuvans.

**6.** Vaccine til anvendelse ifølge krav 5, hvor adjuvansen omfatter DDA/TDB og/eller poly I:C.

5

**7.** Vaccine til anvendelse ifølge et hvilket som helst af de foregående krav, hvor aminosyresekvensen er lipideret for derved at tillade en selv-adjuverende effekt af polypeptidet.

10 **8.** Antigen valgt fra gruppen defineret ifølge et hvilket som helst af kravene 1-4, til anvendelse til behandling af latent tuberkulose.

**9.** Antigen valgt fra gruppen defineret ifølge et hvilket som helst af kravene 1-7, til anvendelse til fremstilling af en vaccine mod reaktivering af latente infektioner

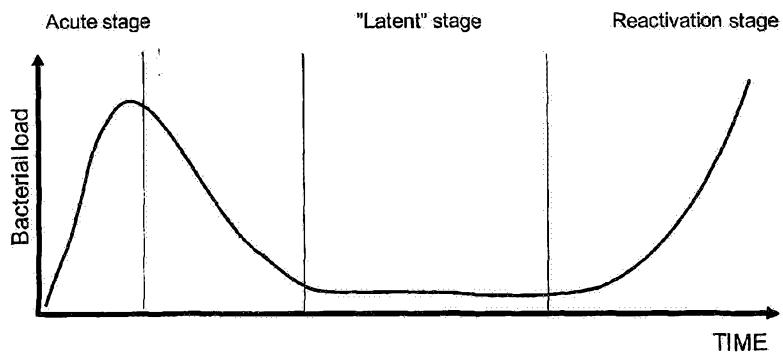
15 forårsaget af arter af tuberkulosekomplekset valgt fra gruppen bestående af *Mycobacterium tuberkulose*, *M.bovis* og *M.africanum*.

**10.** Antigen til anvendelse ifølge krav 9, hvor vaccinen er til indgivelse efter infektion i akut fase og/eller under infektion i latent fase.

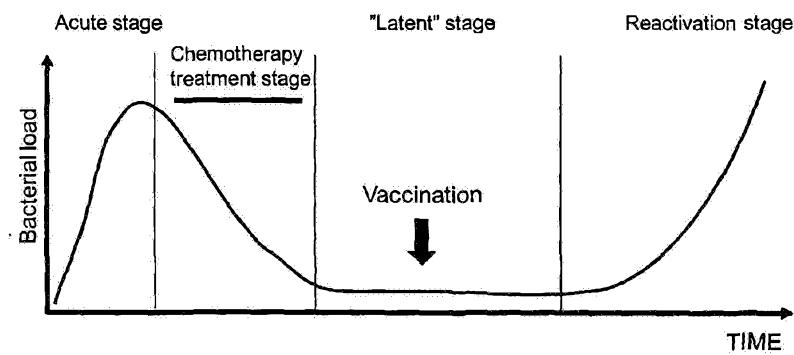
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**11.** Antigen til anvendelse ifølge et hvilket som helst af kravene 9-10, hvor vaccinen omfatter en eller flere immunogene dele som defineret i 1-4.

# DRAWINGS



**Figure 1**



**Figure 2**

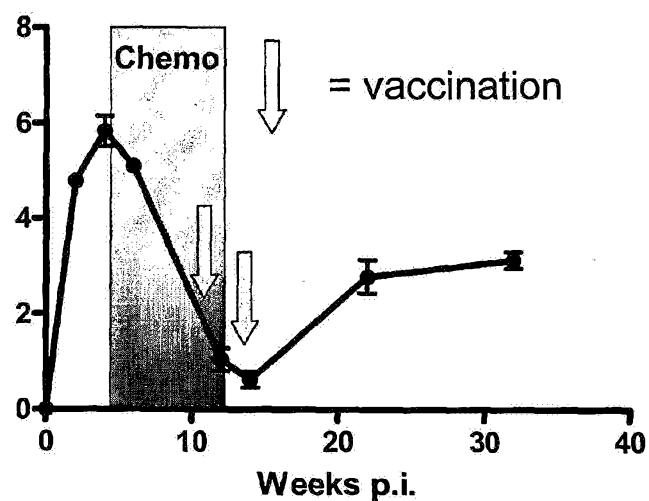


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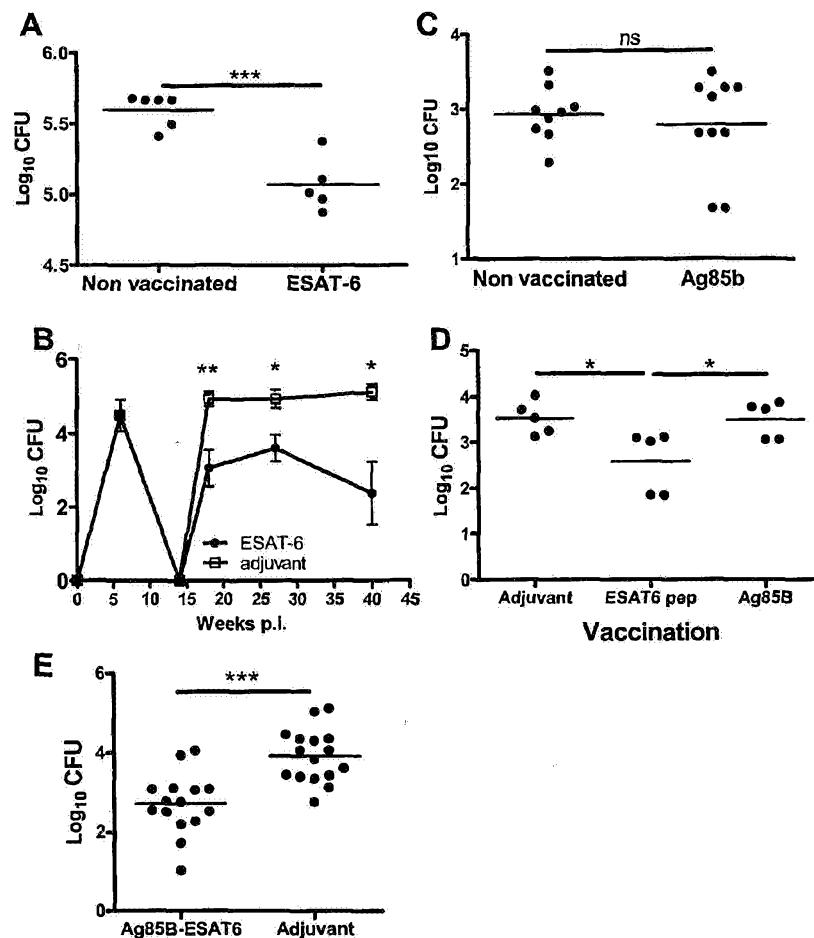


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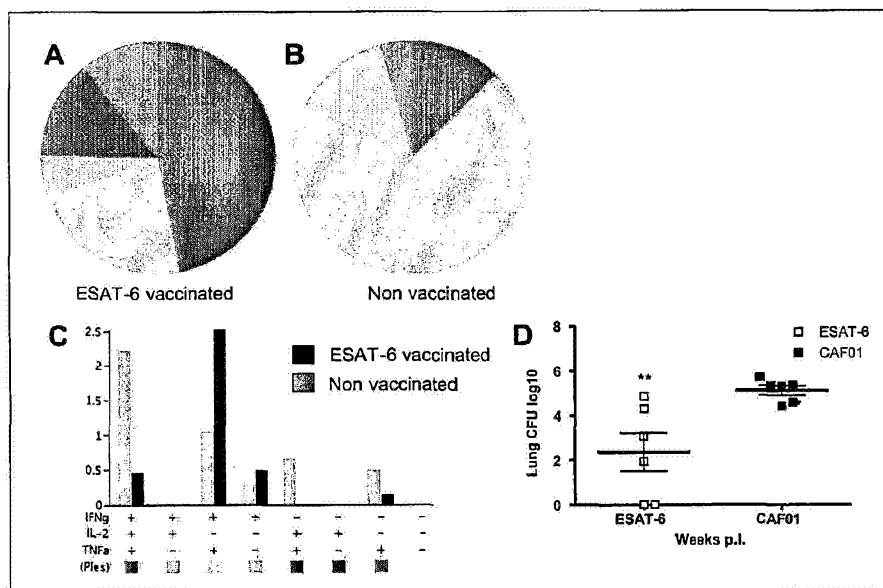


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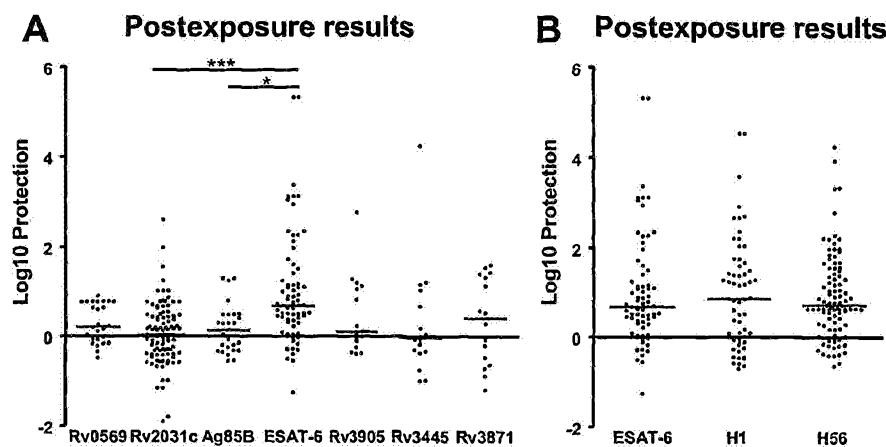


Figure 6

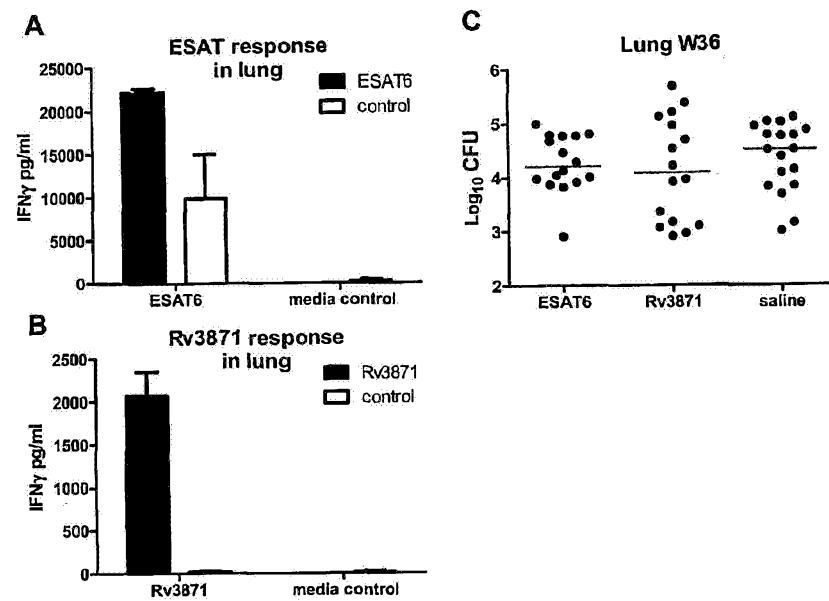


Figure 7