natural course of Mycobacterium infection with additional mycobacterial antigens. The present invention is particularly useful in the context of immunotherapy as stand-alone or as BCG booster for preventive or therapeutic purposes in the Mycobacterium infection field, e.g. preventing Mtb infection and/or prevention of primary TB and/or prevention of reactivation in latently infected subjects. It can also be used in association with standard (e.g. antibiotic-therapy) or any other novel treatment that is currently developed (e.g. small direct or indirect inhibitor molecules; antibodies or immunotherapeutics, etc). The present invention would also be helpful in the veterinary field, for example to reduce or abolish the risk of Mycobacterium infection and/or active disease in animals, especially in bovine and goat breedings.

This technical problem is solved by the provision of the embodiments as defined in the claims.

Other and further aspects, features and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention. These embodiments are given for the purpose of disclosure.

SUMMARY

One aspect of the present disclosure relates to an immunogenic combination comprising or encoding at least two heterooligomeric mycobacterial antigens, and preferably a fusion polypeptide comprising said two heterooligomeric mycobacterial antigens wherein said heterooligomeric mycobacterial antigens are selected from the group of the Esx, PE and PPE antigens and wherein said immunogenic combination comprises or encodes a fusion of the heterooligomeric mycobacterial antigens ESAT6 (Rv3875) with CFP10 (Rv3874).

Another aspect relates to a nucleic acid molecule encoding said combination or fusion of the heterooligomeric mycobacterial antigen and/or said fusion polypeptide of additional mycobacterial antigens comprised or encoded by the immunogenic combination as disclosed herein.

Another aspect relates to a vector comprising one or more nucleic acid molecule(s) as disclosed herein or the nucleic acid molecule(s) comprised in the immunogenic combination as disclosed herein.

Another aspect relates to a host cell comprising the immunogenic combination, the nucleic acid molecule or the vector as disclosed herein.

Another aspect relates to a method for recombinant production of the mycobacterial antigens comprised or encoded by the disclosed immunogenic combination, which comprises the steps of (i) introducing a vector into a host cell to produce a transfected or infected host cell, (ii) culturing *in-vitro* said transfected or infected host cell under conditions suitable for growth of the host cell, (iii) recovering the cell culture, and (iv) optionally, purifying said mycobacterial antigen(s) from the cell culture.

Another aspect relates to a composition comprising at least one of the disclosed immunogenic combination, the nucleic acid molecule, the vector or the host cell or any combination thereof.

Another aspect relates to the use of the disclosed immunogenic combination, the nucleic acid molecule, the vector, the host cell or the composition as described herein for preparing a drug for preventing or treating a Mycobacterium infection or any disease and pathologic condition caused by or associated with such a Mycobacterium infection.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an immunogenic combination comprising or encoding at least two heterooligomeric mycobacterial antigens preferably a fusion polypeptide thereof, which are selected from the group of the Esx, PE and PPE antigens or a nucleic acid molecule encoding said combination/fusion.

Definitions

As used herein throughout the entire application, the terms "a" and "an" are used in the sense that they mean "at least one", "at least a first", "one or more" or "a plurality" of the referenced compounds or steps, unless the context dictates otherwise.

The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

The term "about" or "approximately" as used herein means within 10%, preferably within 8%, and more preferably within 5% of a given value or range.

CLAIMS

1. An immunogenic combination comprising or encoding at least two heterooligomeric mycobacterial antigens, and preferably a fusion polypeptide comprising said two heterooligomeric mycobacterial antigens wherein said heterooligomeric mycobacterial antigens are selected from the group of the Esx, PE and PPE antigens and wherein said immunogenic combination comprises or encodes a fusion of the heterooligomeric mycobacterial antigens ESAT6 (Rv3875) with CFP10 (Rv3874).

~~2.~~1. ~~The immunogenic combination according to claim 1, wherein said heterooligomeric mycobacterial antigens are selected from the group consisting of ESAT-6 (Rv3875), CFP10 (Rv3874), PPE60 (Rv3478), PE31 (Rv3477), TB10.4 (Rv0288) and TB9.8 (Rv0287).~~

~~3.~~2. The immunogenic combination according to claim ~~2~~1, comprising or encoding a ~~fusion of the heterooligomeric mycobacterial antigens ESAT6 (Rv3875) with CFP10 (Rv3874) and preferably a~~ fusion comprising an amino acid sequence having at least 70% of identity with the amino acid sequence shown in SEQ ID NO: 1.

~~4.~~1. ~~The immunogenic combination according to claim 2 or 3, comprising or encoding a fusion of the heterooligomeric mycobacterial antigens TB10.4 (Rv0288) with TB9.8 (Rv0287) and preferably a fusion comprising an amino acid sequence having at least 70% of identity with the amino acid sequence shown in SEQ ID NO: 2.~~

3. The immunogenic combination according to any one of claims 1 to 2, wherein said immunogenic combination comprises two or more fusions of heterooligomeric partners.

4. The immunogenic combination according to claim 3, wherein said immunogenic combination comprises a fusion of CFP10 with ESAT-6 and a fusion of TB10.4 with TB9.8.

5. The immunogenic combination according to any one of claims 1 to 4, wherein said immunogenic combination further comprises or encodes one or more additional mycobacterial antigen(s).

6. The immunogenic combination according to claim 5, wherein said additional mycobacterial antigen(s) is/are selected from the group consisting of antigens of the active, resuscitation and latent phases.

7. The immunogenic combination according to any one of claims 1 to 6, wherein said heterooligomeric mycobacterial antigens and said one or more additional

14. The immunogenic combination according to claim 12 or 13, wherein said fusion polypeptide of additional mycobacterial antigens comprises an amino acid sequence exhibiting at least 70% identity with any of the amino acid sequences shown in SEQ ID NO: 8 to SEQ ID NO: 12.

15. A nucleic acid molecule encoding said combination or fusion of the heterooligomeric mycobacterial antigen and/or said fusion polypeptide of additional mycobacterial antigens comprised or encoded by the immunogenic combination according to any one of claims 1 to 14.

16. The nucleic acid molecule according to claim 15, which exhibits at least 80% identity with any of the nucleotide sequences shown in any of SEQ ID NO: 13 and 15-19.

17. A vector comprising one or more nucleic acid molecule(s) according to claim 15 or 16 or the nucleic acid molecule(s) comprised in the immunogenic combination according to any one of claims 1 to 14.

18. The vector of claim 17, wherein said vector is a plasmid or a viral vector selected from the group consisting of retrovirus, adenovirus, adenovirus-associated virus (AAV), poxvirus, herpes virus, measles virus, foamy virus, alphavirus, vesicular stomatis virus.

19. The vector of claim 18, wherein said vector is a poxvirus vector selected from the group consisting of fowlpox, canarypox and vaccinia virus vector and preferably a vaccinia virus vector selected from the group consisting of Copenhagen, Wyeth, NYVAC and modified Ankara (MVA) strains.

20. The vector according to anyone of claims 17 to 19 which is selected from the group consisting of:

(i) A vector comprising a nucleic acid molecule encoding a fusion comprising heterooligomeric mycobacterial antigens ESAT-6 and CFP10, a nucleic acid molecule encoding Ag85B and a nucleic acid molecule encoding Rv2626; and

26. Use of ~~T~~the immunogenic combination of anyone of claims 1 to 14, the nucleic acid molecule of claim 15 or 16, the vector of anyone of claims 17 to 21, the host cell of claim 22 or the composition of claim 24 or 25 for preparing a drug ~~use~~ for preventing or treating a Mycobacterium infection or any disease and pathologic condition caused by or associated with such a Mycobacterium infection.

27. The ~~immunogenic combination, fusion polypeptide, nucleic acid molecule, vector, host cell or composition~~use according to claim 26, ~~for use~~ for preventing infection or delaying the risk of infection with a Mycobacterium in a subject in need thereof, especially a subject who has been in close contact with an infected individual having developed an active disease.

28. The ~~immunogenic combination, fusion polypeptide, nucleic acid molecule, vector, host cell or composition~~use according to claim 26 or 27, for ~~use for~~ treating an active disease in a subject infected with a Mycobacterium species and especially *M. tuberculosis*.

29. The ~~immunogenic combination, fusion polypeptide, nucleic acid molecule, vector, host cell or composition~~use according to claim 26 or 27, for ~~use for~~ preventing or treating reactivation in a subject latently infected with a Mycobacterium species and especially *M. tuberculosis*.

30. The ~~immunogenic combination, fusion polypeptide, nucleic acid molecule, vector, host cell or composition~~use according to any one of claims 26 to 29, ~~for use~~ as a BCG booster.

31. The ~~immunogenic combination, fusion polypeptide, nucleic acid molecule, vector, host cell or composition~~use according to anyone of claims 26 to 30, ~~for use~~ in association with one or more chemotherapeutic drug(s) effective against a Mycobacterium infection, and especially one or more antibiotic chemotherapy.

32. The ~~immunogenic combination, fusion polypeptide, nucleic acid molecule, vector, host cell or composition~~use according to anyone of claims 26 to 32, ~~for use~~ for inducing or enhancing an immune response in the administered subject.

33. The ~~immunogenic combination, fusion polypeptide, nucleic acid molecule, vector, host cell or composition~~use according to claim 32, wherein said induced or stimulated immune response is a CD4+ and/or CD8+-mediated T cell response directed to a mycobacterial antigen/epitope.