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

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).

In one aspect, the present disclosure relates to a viral vector comprising nucleic acid molecule(s) encoding a fusion of the heterooligomeric mycobacterial antigens ESAT6 with CFP10 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.

The present disclosure also 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.

The present disclosure further 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 viral vector as disclosed herein.

The present disclosure also 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 viral vector or the disclosed host cell or any combination thereof.

Another aspect relates to the use of the viral 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. A viral vector comprising nucleic acid molecule(s) encoding a fusion of the heterooligomeric mycobacterial antigens ESAT6 with CFP10 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.

2. The viral vector according to claim 1, further comprising nucleic acid molecule(s) 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 viral vector according to claim 1 or claim 2, further comprising nucleic acid molecule(s) encoding one or more additional mycobacterial antigen(s), preferably said additional mycobacterial antigen(s) is/are selected from the group consisting of antigens of the active, resuscitation and latent phases.

4. The viral vector according to any one of claims 1 to 3, wherein said heterooligomeric mycobacterial antigens and said one or more additional mycobacterial antigen(s) are independently obtained from a Mycobacterium species of the tuberculosis complex selected from the group consisting of M. tuberculosis (Mtb), M. bovis, M. bovis BCG, M. africanum, M. canetti, M. caprae, and M. microti.

5. The viral vector according to claim 3, wherein said one or more additional mycobacterial antigen(s) is/are selected from the group consisting of Ag85B (Rv1886), RpfB, RpfD, Rv1813, Rv2626 and Rv3407 and a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 3 or 4.

6. The viral vector according to claim 5, comprising nucleic acid molecule(s) encoding a fusion of the heterooligomeric mycobacterial antigens CFP10 and ESAT6 and further expressing additional mycobacterial antigens Rv2626 and Ag85B

7. The viral vector according to claim 5, comprising nucleic acid molecule(s) encoding a fusion of the heterooligomeric mycobacterial antigens CFP10 and ESAT6 and a fusion of the heterooligomeric mycobacterial antigens TB10.4 and TB9.8 and further expressing additional mycobacterial antigens Rv2626, Ag85B, RpfB, RpfD, Rv3407 and Rv1813.

8. The viral vector according to anyone of claims 1 to 7, wherein said additional mycobacterial antigen(s) are encoded in the form of separate polypeptides or in the form of fusion of two, preferably said fusion polypeptide of additional mycobacterial antigens comprises (i) Rv2626 and Ag85B or (ii) RpfB and RpfD or (ii) Rv3407 and Rv1813.

9. The viral vector according to claim 8, wherein said fusion polypeptide of additional mycobacterial antigens comprise one or more peptide(s) to ensure appropriate folding of said mycobacterial antigens such as a protease cleavage site inserted between the upstream and downstream polypeptides of said fusion polypeptide.

10. The viral vector according to claim 8 or 9, 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.

11. The viral vector according to any one of claims 1 to 10, wherein said vector is selected from the group consisting of retrovirus, adenovirus, adenovirus-associated virus (AAV), poxvirus, herpes virus, measles virus, foamy virus, alphavirus, vesicular stomatis virus.

12. The viral vector of claim 11, wherein said viral 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.

13. The viral vector according to anyone of claims 1 to 12 which is selected from the group consisting of:

(i) A viral 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

(ii) A viral vector comprising a nucleic acid molecule encoding a fusion comprising heterooligomeric mycobacterial antigens ESAT-6 and CFP10; a nucleic acid molecule encoding a fusion comprising heterooligomeric mycobacterial antigens TB10.4 and TB9.8, a nucleic acid molecule encoding a fusion polypeptide comprising the additional mycobacterial antigens Rv2626 and Ag85B; a nucleic acid molecule encoding a fusion polypeptide comprising the additional mycobacterial antigens RpfB and RpfD; and a nucleic acid molecule encoding a fusion polypeptide comprising the additional mycobacterial antigens Rv3407 and Rv1813.

14. The viral vector according to claim any one of claims 1 to 13, wherein said nucleic acid molecule(s) is placed under the transcriptional control of a promoter suitable for ensuring expression of the encoded fusion (s) and additional mycobacterial antigen in a mammalian cell and in particular under the transcriptional control of a vaccinia promoter preferably selected from the group consisting of p7.5K, pH5R, pB2R, pSE/L and pA35R promoters.

15. A host cell comprising the viral vector according to anyone of claims 1 to 14.

16. A composition comprising at least one of the viral vector of anyone of claims 1 to 14 or the host cell of claim 15 or any combination thereof.

17. The composition of claim 16 which further comprises a pharmaceutically acceptable vehicle.

18. Use of the viral vector of anyone of claims 1 to 14, the host cell of claim 15 or the composition of claim 16 or 17 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.

19. The use according to claim 18, 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.

20. The use according to claim 18 or 19, for treating an active disease in a subject infected with a Mycobacterium species and especially M. tuberculosis.

21. The use according to claim 18 or 19, for preventing or treating reactivation in a subject latently infected with a Mycobacterium species and especially M. tuberculosis.

22. The use according to any one of claims 18 to 21, as a BCG booster.

23. The use according to anyone of claims 18 to 22, in association with one or more chemotherapeutic drug(s) effective against a Mycobacterium infection, and especially one or more antibiotic chemotherapy.

24. The use according to anyone of claims 18 to 23, for inducing or enhancing an immune response in the administered subject.

25. The use according to claim 24, wherein said induced or stimulated immune response is a CD4+ and/or CD8+-mediated T cell response directed to a mycobacterial antigen/epitope.