detect due to the paucity of immunodominant antigens. Currently, the most widely used biomarkers are based on elevated frequencies of CD4 T cells producing IFNγ. Increasing evidence questions the value of IFNγ as correlate of protection in TB (9,10). Undoubtedly IFNγ does play a crucial role in defense against MTB (11), but determination of IFNγ alone can no longer be considered as a reliable marker of protective immunity.

A recombinant BCG strain expressing a phagolysosomal escape domain is described in WO99/101496, the content of which is herein incorporated by reference. The phagolysosomal escape domain enables the strain to escape from the phagosome of infected host cells by perforating the membrane of the phagosome. In order to provide an acidic phagosomal pH for optimal phagolysosomal escape activity, a urease-deficient recombinant strain was developed. This strain is disclosed in WO2004/094469, the content of which is herein incorporated.

A recombinant Δ*ure*C *Hly*$^+$ rBCG (rBCG) strain expressing membrane-perforating listeriolysin (Hly) of *Listeria monocytogenes* and devoid of urease C induces superior protection against aerogenic challenge with MTB as compared to parental BCG (pBCG) in a preclinical model (12). This vaccine construct has successfully proven safety and immunogenicity in a phase I clinical trial (US 61/384,375), the content of which is herein incorporated by reference.

In the present study, it is shown that rBCG and pBCG induce marked Th1 immune responses, whilst only rBCG elicits are profound Th17 response in addition. It was also observed earlier recruitment of antigen-specific T lymphocytes to the lung upon MTB infection of rBCG-vaccinated mice. These T cells produced abundant Th1 cytokines after restimulation. Superior protective efficacy of rBCG was apparently dependent on IL17. Elevated IL17 production after rBCG, but not pBCG vaccination, was also detected in healthy volunteers during a phase I clinical trial. Our findings identify a general immunologic pathway as a marker for improved vaccination

CLAIMS:

1. Use of (a) a reagent for restimulating immune cells from a previously vaccinated subject, and (b) a reagent for detecting a Th17 immune response in manufacture of a reagent kit for determining efficacy of a vaccine, wherein the determining efficacy of a vaccine comprises determining the Th17 immune response in a vaccinated subject, wherein a detectable Th17 immune response is indicative for protective immunity in said subject.

2. The use of claim 1, wherein the vaccine is a live vaccine, particularly a mycobacterial live vaccine.

3. The use of claim 2, wherein the vaccine is a recombinant Mycobacterium which comprises a recombinant nucleic acid molecule encoding a fusion polypeptide comprising (a) a domain capable of eliciting an immune response and (b) a phagolysosomal escape domain.

4. The use of claim 3, wherein the Mycobacterium is urease-deficient.

5. The use of claim 4, wherein the Mycobacterium is rBCGΔUreC :: Hly^+ :: Hyg^+.

6. The use of claim 5 , wherein the vaccine is a subunit vaccine.

7. The use of any one of claims 1-6, wherein the vaccine is a vaccine against mycobacterial infections, particularly pulmonary mycobacterial infections, more particularly tuberculosis.

8. The use of any one of claims 1-7, wherein the reagent for restimulating immune cells from a previously vaccinated subject is a mycobacterial antigen.

9 . The use of any one of claims 1-8 , wherein determining the Th17 immune response comprises subjecting a sample comprising immune cells from said vaccinated subject to a restimulation with an immunogen corresponding to the immunogen in said vaccine and determining the presence and/or amount of Th17 immune response associated cells and/or in said sample.

10. The use of any one of claims 1-9, wherein the reagent for detecting a Th17 immune response is an immunological detection reagent specific for a cell-surface marker and/or a cytokine present in and/or secreted by a cell associated with the Th17 response.

11. The use of claim 10, wherein the immunological detection reagent carries a labelling group.

12 . The use of any one of claims 1-11 , wherein determining the Th17 immune response comprises determining IL17, e.g. by immunological methods.

13 . The use of any one of claims 1-12 , wherein the subject is a mammal, e·g. a human.

14 . The use of any one of claims 1-13 , wherein the Th17 immune response is determined 20-50 days after vaccination.

15 . A reagent kit for determining efficacy of a vaccine, comprising (a) a reagent

for restimulating immune cells from a previously vaccinated subject, and (b) a reagent for detecting a Th17 immune response.

16. The reagent kit of claim 15, wherein the vaccine is a live vaccine, particularly a mycobacterial live vaccine.

17. The reagent kit of claim 16, wherein the vaccine is a recombinant Mycobacterium which comprises a recombinant nucleic acid molecule encoding a fusion polypeptide comprising
a. a domain capable of eliciting an immune response and
b. a phagolysosomal escape domain.

18. The reagent kit of claim 17, wherein the Mycobacterium is urease-deficient.

19. The reagent kit of claim 18, wherein the Mycobacterium is rBCGΔUreC :: Hly^+ :: Hyg^+.

20. The reagent kit of claim 15, wherein the vaccine is a subunit vaccine.

21. The reagent kit of any one of claims 15-20, wherein the vaccine is a vaccine against mycobacterial infections, particularly pulmonary mycobacterial infections, more particularly tuberculosis.

22. The reagent kit of any one of claims 15-21, wherein the reagent for restimulating immune cells from a previously vaccinated subject is a mycobacterial antigen.

23. The reagent kit of anyone of claims 15-22, wherein the reagent for detecting a Th17 immune response is an immunological detection reagent specific for a cell-surface marker and/or a cytokine present in and/or secreted by a cell associated with the Th17 response.

24. The reagent kit of claim 23, wherein the cytokine is IL-17.

25. The reagent kit of claim 23, wherein the immunological detection reagent carries a labelling group.

detect due to the paucity of immunodominant antigens. Currently, the most widely used biomarkers are based on elevated frequencies of CD4 T cells producing IFNγ. Increasing evidence questions the value of IFNγ as correlate of protection in TB (9,10). Undoubtedly IFNγ does play a crucial role in defense against MTB (11), but determination of IFNγ alone can no longer be considered as a reliable marker of protective immunity.

A recombinant BCG strain expressing a phagolysosomal escape domain is described in WO99/10496, the content of which is herein incorporated by reference. The phagolysosomal escape domain enables the strain to escape from the phagosome of infected host cells by perforating the membrane of the phagosome. In order to provide an acidic phagosomal pH for optimal phagolysosomal escape activity, a urease-deficient recombinant strain was developed. This strain is disclosed in WO2004/094469, the content of which is herein incorporated.

A recombinant Δ*ure*C *Hly*$^{+}$ rBCG (rBCG) strain expressing membrane-perforating listeriolysin (Hly) of *Listeria monocytogenes* and devoid of urease C induces superior protection against aerogenic challenge with MTB as compared to parental BCG (pBCG) in a preclinical model (12). This vaccine construct has successfully proven safety and immunogenicity in a phase I clinical trial (US 61/384,375), the content of which is herein incorporated by reference.

In the present study, it is shown that rBCG and pBCG induce marked Th1 immune responses, whilst only rBCG elicits a profound Th17 response in addition. It was also observed earlier recruitment of antigen-specific T lymphocytes to the lung upon MTB infection of rBCG-vaccinated mice. These T cells produced abundant Th1 cytokines after restimulation. Superior protective efficacy of rBCG was apparently dependent on IL17. Elevated IL17 production after rBCG, but not pBCG vaccination, was also detected in healthy volunteers during a phase I clinical trial. Our findings identify a general immunologic pathway as a marker for improved vaccination

CLAIMS:

1. Use of (a) a reagent for restimulating immune cells from a previously vaccinated subject, and (b) a reagent for detecting a T helper type 17 (Th17) immune response in manufacture of a reagent kit for determining efficacy of a recombinant mycobacterial live vaccine, wherein the determining efficacy of a vaccine comprises determining the Th17 immune response in a vaccinated subject, wherein a detectable Th17 immune response is indicative for protective immunity in said subject.

2. The use of claim 1, wherein the vaccine is a recombinant Mycobacterium which comprises a recombinant nucleic acid molecule encoding a fusion polypeptide comprising (a) a domain capable of eliciting an immune response and (b) a phagolysosomal escape domain.

3. The use of claim 2, wherein the Mycobacterium is urease-deficient.

4. The use of claim 3, wherein the Mycobacterium is rBCGΔUreC :: Hly^+:: Hyg^+.

5. The use of claim 4, wherein the vaccine is a subunit vaccine.

6. The use of any one of claims 1-5, wherein the vaccine is a vaccine against mycobacterial infections, particularly pulmonary mycobacterial infections, more particularly tuberculosis.

7. The use of any one of claims 1-6, wherein the reagent for restimulating immune cells from a previously vaccinated subject is a mycobacterial antigen.

8. The use of any one of claims 1-7, wherein determining the Th17 immune response comprises subjecting a sample comprising immune cells from said vaccinated subject to a restimulation with an immunogen corresponding to the immunogen in said vaccine and determining the presence and/or amount of Th17 immune response associated cells in said sample.

9. The use of any one of claims 1-8, wherein the reagent for detecting a Th17 immune response is an immunological detection reagent specific for a cell-surface marker and/or a cytokine present in and/or secreted by a cell associated with the Th17 response.

10. The use of claim 9, wherein the immunological detection reagent carries a labelling group.

11. The use of any one of claims 1-10, wherein determining the Th17 immune response comprises determining IL17, e.g. by immunological methods.

12. The use of any one of claims 1-11, wherein the subject is a mammal, eg. a human.

13. The use of any one of claims 1-12, wherein the Th17 immune response is determined 20-50 days after vaccination.

14. A reagent kit for determining efficacy of a recombinant mycobacterial live vaccine, comprising (a) a reagent for restimulating immune cells from a previously vaccinated subject, and (b) a reagent for detecting a Th17 immune response.

15. The reagent kit of claim 14, wherein the vaccine is a recombinant Mycobacterium which comprises a recombinant nucleic acid molecule encoding a fusion polypeptide comprising
    a. a domain capable of eliciting an immune response and
    b. a phagolysosomal escape domain.

16. The reagent kit of claim 15, wherein the Mycobacterium is urease-deficient.

17. The reagent kit of claim 16, wherein the Mycobacterium is rBCGΔUreC :: Hly^+:: Hyg^+.

18. The reagent kit of claim 13, wherein the vaccine is a subunit vaccine.

19. The reagent kit of any one of claims 13-18, wherein the vaccine is a vaccine against mycobacterial infections, particularly pulmonary mycobacterial infections, more particularly tuberculosis.

20. The reagent kit of any one of claims 13-19, wherein the reagent for restimulating immune cells from a previously vaccinated subject is a mycobacterial antigen.

21. The reagent kit of any one of claims 13-20, wherein the reagent for detecting a Th17 immune response is an immunological detection reagent specific for a cell-surface marker and/or a cytokine present in and/or secreted by a cell associated with the Th17 response.

22. The reagent kit of claim 21, wherein the cytokine is IL-17.

23. The reagent kit of claim 21, wherein the immunological detection reagent carries a labelling group.