T cell and antibody responses against antigens encoded by recombinant poxviruses, such as MVA. The invention fulfills this need.

Brief Summary of the Invention

[0028] The invention is defined in the independent claims. Some optional features of the invention are defined in the dependent claims. As disclosed herein, there is a recombinant modified Vaccinia Ankara (MVA) virus comprising a Pr13.5 promoter linked to a nucleotide sequence encoding a neoantigen and uses thereof. In one example, a method of inducing a robust CD8 T cell response against a neoantigen in mammal, preferably a human, comprising administering one or more immunizations of the MVA virus to the mammal, including a human.

[0029] In various embodiments, the Pr13.5 promoter comprises at least 1 copy of a nucleic acid sequence of at least 40 bases having at least 95%, 98%, or 100% identity with SEQ ID NO:1.

[0030] In various embodiments, the Pr13.5 promoter comprises at least 1 copy of a second nucleotide sequence of at least 31 nucleotides that has at least 95%, 98% or 100% identity with SEQ ID NO:1.

[0031] In various embodiments, the Pr13.5 promoter comprises 2 copies of a nucleotide sequence of at least 40 nucleotides that has 100% identity with SEQ ID NO:1.

[0032] In various embodiments, the Pr13.5 promoter comprises SEQ ID NO:2.

Brief Description of the Drawings

[0033] Figure 1 depicts the upstream sequence of the MVA013.5L gene (SEQ ID NO:3). Sequences of the Pr13.5-short and Pr13.5-long promoters are given. Dashed line: Pr13.5-long (Pos. 15878-15755). Solid line: Pr13.5-short (Pos. 15808-15755). Underlined: ATG start codon of

[0077] The invention encompasses use of the recombinant poxviruses of the invention as vaccines, particularly for the treatment or prevention of infections and diseases of a mammal, including a human.

Kits comprising recombinant MVA

[0078] The invention provides kits comprising the recombinant poxviral vector, preferably MVA virus, according to the present invention. The kit can comprise at least one, two, three, four, or more containers or vials of the recombinant poxviral vector, preferably MVA virus, together with instructions for the administration of the virus to a mammal, including a human. The instructions can indicate that the recombinant virus is administered to the mammal, preferably a human, in one or multiple (i.e., 2, 3, 4, 5, 6, etc.) dosages at specific timepoints (e.g., at least 4 weeks, at least 6 weeks, at least 8 weeks after the previous administration). Preferably, the instructions indicate that the recombinant virus is to be administered to a mammal, preferably a human, in at least 1, at least 2, at least 3, or at least 4 dosages.

Methods of Inducing a CD8 T Cell and/or Antibody Response

[0079] The invention encompasses methods of inducing a CD8 T cell and/or antibody response in a host. In preferred embodiments, the method comprises administering at least one, two, three, four, or five immunizations of a recombinant poxvirus, preferably MVA, comprising a Pr13.5 promoter to the mammal, including a human.

Administration to a Host

[0080] The recombinant poxvirus, preferably MVA, can be used for the treatment of a wide range of mammals including

humans and even immune-compromised humans. Hence, a pharmaceutical composition and also a vaccine for inducing an immune response in a mammal, including a human can also be provided.

[0081] The vaccine preferably comprises the recombinant poxvirus, preferably MVA, in a concentration range of 10^4 to 10^9 TCID (tissue culture infectious dose) $_{50}$/ml, preferably in a concentration range of 10^5 to $5x10^8$ $TCID_{50}$/ml, more preferably in a concentration range of 10^6 to 10^8 $TCID_{50}$/ml, and most preferably in a concentration range of 10^7 to 10^8 $TCID_{50}$/ml, especially 10^8 $TCID_{50}$/ml.

[0082] A preferred vaccination dose for mammal, preferably a human, comprises 10^6 to 10^9 $TCID_{50}$, most preferably a dose of 10^7 $TCID_{50}$ or 10^8 $TCID_{50}$, especially 10^8 $TCID_{50}$.

[0083] The pharmaceutical composition may generally include one or more pharmaceutically acceptable and/or approved carriers, additives, antibiotics, preservatives, adjuvants, diluents and/or stabilizers. Such auxiliary substances can be water, saline, glycerol, ethanol, oil, wetting or emulsifying agents, pH buffering substances, or the like. Suitable carriers are typically large, slowly metabolized molecules such as proteins, polysaccharides, polylactic acids, polyglycollic acids, polymeric amino acids, amino acid copolymers, lipid aggregates, or the like.

[0084] For the preparation of vaccines, the recombinant poxvirus, preferably MVA, according to the invention can be converted into a physiologically acceptable form. This can be done based on the experience in the preparation of poxvirus vaccines used for vaccination against smallpox (as described by Stickl et al. 1974).

[0085] For example, the purified virus can be stored at -80℃ with a titre of $5x10^8$ $TCID_{50}$/ml formulated in about 10 mM Tris, 140 mM NaCl pH 7.4. For the preparation of vaccine shots, e.g., 10^2-10^8 particles of the virus can be lyophilized in 100 µl to 1 ml of phosphate-buffered saline (PBS) in the presence of 2% peptone and 1% human albumin in an ampoule, preferably a glass ampoule. Alternatively, the vaccine shots can be produced by stepwise freeze-drying of the virus in a formulation. This formulation can contain additional additives such as mannitol, dextran, sugar, glycine, lactose or polyvinylpyrrolidone or other aids such as antioxidants or inert gas, stabilizers or recombinant proteins (e.g. human serum albumin) suitable for in vivo administration. The glass ampoule is then sealed and can be stored between 4 ℃ and room temperature for several months. However, as long as no need exists the ampoule is stored preferably at temperatures below -20 ℃.

[0086] For vaccination or therapy, the lyophilisate can be dissolved in an aqueous solution, preferably physiological saline or Tris buffer, and administered either systemically or locally, i.e. parenteral, subcutaneous, intravenous, intramuscular, intranasal, or any other path of administration know to the skilled practitioner. The mode of administration, the dose and the number of administrations can be optimized by those skilled in the art in a known manner. However, most commonly a mammal, preferably a human, is vaccinated with a second administration about two weeks to six weeks after the first vaccination administration. Third, fourth, and subsequent administrations will most commonly be about two weeks to six weeks after the previous administration.

[0087] Methods for immunizing mammals,

including a human may also be provided. In one embodiment a subject mammal, which includes rats, rabbits, mice, and humans are immunized comprising administering a dosage of a recombinant MVA to the mammal, preferably to a human. In one embodiment, the first dosage comprises 10^8 $TCID_{50}$ of the recombinant MVA virus and the second and additional dosages (i.e., third, fourth, fifth, etc.) comprise 10^8 $TCID_{50}$ of the virus. The administrations can be in a first (priming) dose and a second, or further, (boosting) dose(s).

[0088] The immunization can be administered either systemically or locally, i.e. parenterally, subcutaneously, intravenously, intramuscularly, intranasally, or by any other path of administration known to the skilled practitioner.

CD8 T cell and antibody responses

[0089] Immunizations with the recombinant MVA of the invention can induce a robust CD8 T cell response. In preferred embodiments, after the first, second, third, fourth, fifth, etc. immunization, the recombinant MVA induces a robust CD8 T cell response in the mammal, preferably a human, against the encoded antigen that is greater than the CD8 T cell response against the immunodominant viral CD8 T cell epitope, e.g. TSYKFESV (SEQ ID NO:5) encoded by the MVA vector. Preferably, after the second, third, fourth, fifth, etc. immunization, an immunodominant T cell response is induced in the mammal, preferably a human, against the encoded antigen. Preferably, after the second, third, fourth, fifth, etc. immunization, the recombinant MVA induces a CD8 T cell response in the mammal, preferably a human, against the encoded antigen that is at least 10%, 15%, 20%, 25%, 30%, or 35% of total CD8 T cells. Preferably, after the second, third, fourth, fifth, etc.

CLAIMS

We claim:

1. The use of a recombinant modified Vaccinia Ankara (MVA) virus for the manufacture of a medicament for inducing a robust CD8 T cell response against a neoantigen in a human;

wherein the recombinant MVA comprises a Pr13.5 promoter operably linked to a nucleotide sequence encoding the neoantigen,

wherein the Pr13.5 promoter comprises 2 copies of a nucleotide sequence of at least 40 nucleotides that has 100% identity with SEQ ID NO:1.

2. The use of claim 1, wherein the Pr13.5 promoter comprises at least 1 copy of a nucleic acid sequence of at least 40 bases having at least 98% identity with SEQ ID NO:1.

3. The use of claim 1, wherein the Pr13.5 promoter comprises at least 1 copy of a nucleic acid sequence of at least 40 bases having 100% identity with SEQ ID NO:1.

4. The use of claim 1, wherein the Pr13.5 promoter comprises at least 1 copy of a second nucleotide sequence of at least 31 nucleotides that has at least 98% identity with SEQ ID NO:1.

5. The use of claim 1, wherein the Pr13.5 promoter comprises at least 1 copy of a second nucleotide sequence of at least 31 nucleotides that has 100% identity with SEQ ID NO:1.

6. The use of claim 1, wherein the Pr13.5 promoter comprises SEQ ID NO:2.

7. A recombinant modified Vaccinia Ankara (MVA) virus comprising a Pr13.5 promoter operably linked to a nucleotide sequence encoding a neoantigen,

wherein the Pr13.5 promoter comprises 2 copies of a nucleotide sequence of at least 40 nucleotides that has 100% identity with SEQ ID NO:1.

8. The recombinant MVA of claim 7, wherein the Pr13.5 promoter comprises at least 1 copy of a nucleic acid sequence of at least 40 bases having at least 98% identity with SEQ ID NO:1.

9. The recombinant MVA of claim 7, wherein the Pr13.5 promoter comprises at least 1 copy of a nucleic acid sequence of at least 40 bases having 100% identity with SEQ ID NO:1.

10. The recombinant MVA of claim 7, wherein the Pr13.5 promoter comprises at least 1 copy of a second nucleotide sequence of at least 31 nucleotides that has at least 98% identity with SEQ ID NO:1.

11. The recombinant MVA of claim 7, wherein the Pr13.5 promoter comprises at least 1 copy of a second nucleotide sequence of at least 31 nucleotides that has 100% identity with SEQ ID NO:1.

12. The recombinant MVA of claim 7, wherein the Pr13.5 promoter comprises SEQ ID NO:2.