innate and adaptive immune response of the host. Among the known poxviral factors counteracting the interferon system are the secreted receptor-like proteins B19 and B8 that bind and neutralize type I and type II interferons, respectively. Other poxviral proteins such as VACV E3, K7, C6, N1, C7, K1, K3 and H1 inhibit the induction of type I interferon or block interferon signaling and effector pathways. The fact that poxviruses encode such a multitude of proteins to counteract the interferon system highlights the importance of this system of innate immune defense for the control and eventual clearance of poxviruses by the infected host organism. Apart from the interferon system, orthopoxviruses encode other immune-modulating proteins interfering with induction or function of cytokines like IL-1β, IL-18, and of chemokines including VACV B16R, WR013, C23L/vCCI. These cytokines are also important in restricting pathogen spread and protecting the host from severe pathogen-induced damage. Poxviral proteins subverting pattern recognition pathways are universally efficient in thwarting induction of IFNs as well as of other cytokines and chemokines.

[010] Accordingly, it is a primary objective of the invention to enhance recognition of poxviruses by cellular dsRNA sensors, as well as to increase the innate immune activation induced by MVA. Disclosed herein are recombinant poxviruses engineered to produce dsRNA early in infection by inserting two partially identical DNAs at two separate locations in the genome. Those DNAs are operable linked to strong early promoters oriented such that one DNA produces a ‘sense’ transcript and the other produces a complementary ‘antisense’ transcript. The two partially or completely complementary transcripts subsequently anneal to produce dsRNA. Efficient induction of IFN-β, an important marker of innate immune activation, was observed with early dsRNAs over a wide size range, with decreasing efficiencies as the complementary transcripts were shortened down to 50 bp.

SUMMARY

[011] The invention encompasses a recombinant poxvirus comprising heterologous nucleic acids expressing excess double-stranded RNA (dsRNA) early in infection. In one embodiment, the invention encompasses a method of enhancing innate immune activation comprising administering the recombinant poxvirus to a vertebrate subject, wherein said administration enhances production of type I interferons (type I IFNs), cytokines and chemokines in the subject.

[012] In a further embodiment, the recombinant poxvirus transcribes sense and antisense RNAs from both strands of a native poxvirus sequence, preferably an early gene, more preferably an immediate-early gene. .

[013] In a further embodiment, the recombinant poxvirus transcribes heterologous nucleic acid as sense and antisense RNAs from both strands of the heterologous sequence.

[014] In a further embodiment, the invention encompasses a method of attenuating standard replication-competent vaccinia virus strains and of other species derived from chordopoxvirinae by expression of excess early dsRNA.

[015] In various embodiments, the poxvirus further comprises heterologous sequences encoding one or more costimulatory molecules.

[016] In various embodiments, the poxvirus further comprises heterologous sequences encoding one or more bacterial, viral, fungal, parasite, or tumor antigens.

[017] In various embodiments, the poxvirus is an orthopoxvirus, a parapoxvirus, a yatapoxvirus, an avipoxvirus, a leporipoxvirus, a suipoxvirus, a capripoxvirus, a cervidpoxvirus, or a molluscipoxvirus. The orthopoxvirus may be selected from the group consisting of vaccinia virus, cowpox virus, and monkeypox virus. The vaccinia virus may be a modified vaccinia virus Ankara (MVA), e.g. modified vaccinia virus Ankara Bavarian Nordic (MVA-BN).

[018] In various embodiments, the heterologous or endogenous nucleic acids generating dsRNA comprise sequences encoding partially or completely complementary RNA

transcripts, wherein the complementary portions of RNA transcripts anneal after transcription to form dsRNA.

[019] In various embodiments, the heterologous nucleic acids encoding completely or partially complementary RNA transcripts are identical within the complementary region, or have a similarity of more than 99%, of more than 95%, of more than 90%, of more than 80%, or of more than 70% within the complementary region.

[020] Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[021] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

[022] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate one (several) embodiment(s) of the invention and together with the description, serve to explain the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[023] **Figure 1** depicts a schematic representation of CVA mutants overproducing early dsRNA and corresponding control mutants. Boxes represent CVA ORFs in the genomic region between ORFs B14R and B20R and are not drawn to scale. The box representing the CVA version of the B15 ORF is shown in black, whereas the MVA version of B15R is indicated by a diamond pattern. A hatched box represents the B19R ORF. All other ORFs are shown as grey boxes. The bacterial selection markers (neo^r and zeo^r) replacing CVA ORFs in the various deletion mutants are shown as smaller boxes with an indication of the specific marker.

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[050] As used herein, the singular forms “a”, “an”, and “the” include plural references unless the context clearly indicates otherwise. Thus, for example, reference to “an epitope” includes reference to one or more epitopes and reference to “the method” includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods provided herein.

[051] Unless otherwise indicated, the term “at least” preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention provided herein. Such equivalents are encompassed by the present invention.

[052] Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising” mean “includes”, and therefore include a stated integer or step or group of integers or steps and do exclude any other integer or step or group of integers or steps. When used herein the term “comprising” can be substituted with the term “containing”, “including” or “having”. Any of the aforementioned terms (comprising, containing, including, having), whenever used herein in the context of an aspect or embodiment of the present invention can be substituted with the term “consisting of”.

[053] When used herein, the term “consisting of” excludes any element, step, or ingredient not specified in the claim. When used herein, “consisting essentially of” excludes any materials or steps “which would affect the basic and novel characteristics” of the product or

[067] **CD4**. Cluster of differentiation factor 4, a T-cell surface protein that mediates interaction with the MHC Class II molecule. Cells that express CD4, referred to as "CD4+" cells, are often helper T (*e.g.*, "T_H", "T_H1" or "T_H2") cells.

[068] **CD8**. Cluster of differentiation factor 8, a T-cell surface protein that mediates interaction with the MHC Class I molecule. Cells that express CD8, referred to as "CD8+" cells, are often cytotoxic T ("CTL") cells.

[069] **Costimulatory molecule**. T-cell activation typically requires binding of the T-cell receptor ("TCR") with a peptide-MHC complex as well as a second signal delivered via the interaction of a costimulatory molecule with its ligand. Costimulatory molecules are molecules that, when bound to their ligand, deliver the second signal required for T-cell activation. The most well-known costimulatory molecule on the T-cell is CD28, which binds to either B7-1 or B7-2. Other costimulatory molecules that can also provide the second signal necessary for activation of T-cells include intracellular adhesion molecule-1 ("ICAM-1"), intracellular adhesion molecule-2 ("ICAM-2"), leukocyte function associated antigen-1 ("LFA-1"), leukocyte function associated antigen-2 ("LFA-2"), and leukocyte function associated antigen-3 ("LFA-3"). The combination of B7-1, ICAM-1, and LFA-3 is referred to as "TRICOM", for "TRIad of COstimulatory Molecules".

[070] **Dendritic cell (DC)**. Dendritic cells are the main antigen presenting cells ("APCs") involved in primary immune responses. Dendritic cells include plasmacytoid dendritic cells and myeloid dendritic cells. Their major function is to obtain antigen in tissues, migrate to lymphoid organs and present the antigen in order to activate T-cells. Immature dendritic cells originate in the bone marrow and reside in the periphery as immature cells.

[071] **Double-stranded RNA (dsRNA).** Recombinant poxvirus virus comprising heterologous or native nucleic acids expressing an increased number or amount of dsRNA (e.g. expressing excess dsRNA) according to any embodiment of the present invention triggers the release of cytokines, chemokines, effector molecules and/or increased expression of

costimulatory molecules or activates one or more pattern recognition receptor(s) (PRRs) and/or activates cells (e.g dendritic cells, macrophages, B cells or other types of immune cells) and thus preferably triggers or induces an enhanced innate immune response. Excess dsRNA can be determined by any method suitable, preferably by using a method to determine enhanced innate immune response or any method as described in the examples preferably the method according to Example 16. Preferably, excess dsRNA can be defined as the amount of dsRNA early in infection that generates an enhanced innate immune response when using the recombinant poxvirus vector of the present invention. More preferably, excess dsRNA can be defined as the amount of dsRNA transcribed during the early phase of virus infection with the recombinant poxvirus comprising heterologous nucleic acids expressing or generating dsRNA compared to the control. Excess dsRNA can be generated by driving transcription of sense and antisense RNA with identical or overlapping sequence stretches preferably of at least 100 bp in length by using poxviral promoters with early activity (e,g, immediate early, early, or early/late poxviral promoters). Another method of determining excess dsRNA is to determine enhanced activation of one or more PRR(s) e.g. PKR as determined by increased phosphorylation of a PKR substrate (e.g. eIF2α at position Serine-51) as shown in Example 7. Preferably, increased phosphorylation of a PKR substrate (e.g. eIF2α at position Serine-51) increases by at least 2-fold, at least 4-fold, at least 6-fold, at least 8-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 100-fold or more as compared to the control.

[072] **Early in infection**. Gene expression of poxviruses is regulated in a cascade fashion. Early transcription of viral genes is driven by the viral transcriptional machinery carried with the viral particle into the newly infected cell and is under control of early viral promoters that are recognized by the early viral transcription complex. It is well known that once the virion and host membranes have fused and the virus core has been released into the cytoplasm, the endogenous RNA polymerase and encapsidated transcription factors that comprise the viral

WE CLAIM:

1. A recombinant poxvirus comprising heterologous nucleic acids expressing excess double-stranded RNA (dsRNA) early in infection.

2. The recombinant poxvirus of claim 1, further comprising heterologous sequences encoding one or more costimulatory molecules.

3. The recombinant poxvirus of claim 2, wherein the one or more costimulatory molecules is TRICOM (B7-1, ICAM-1, and LFA-3).

4. The recombinant poxvirus of any one of claims 1 to 3, further comprising heterologous sequences encoding one or more bacterial, viral, fungal, parasite, or tumor antigens.

5. The recombinant poxvirus of any one of claims 1 to 4, wherein the poxvirus is an orthopoxvirus, a parapoxvirus, a yatapoxvirus, an avipoxvirus, a leporipoxvirus, a suipoxvirus, a capripoxvirus, a cervidpoxvirus, or a molluscipoxvirus..

6. The recombinant poxvirus of claim 5, wherein the orthopoxvirus is selected from the group consisting of vaccinia virus, cowpox virus, and monkeypox virus.

7. The recombinant poxvirus of claim 6, wherein the vaccinia virus is selected from the group consisting of vaccinia virus-Western Reserve, vaccinia virus-Copenhagen, Dryvax (also known as vaccinia virus-Wyeth), vaccinia virus Lister, vaccinia virus Acambis 2000 and 3000, rabbitpox virus, buffalopox virus, modified vaccinia virus Ankara (MVA), and modified vaccinia virus Ankara-Bavarian Nordic (MVA-BN).

8. The recombinant poxvirus of any one of claims 1 to 7, wherein the heterologous nucleic acids generating dsRNA comprise sequences encoding partially or completely complementary RNA transcripts, wherein the complementary portions of RNA transcripts anneal after transcription to form dsRNA.

9. The recombinant poxvirus of claim 8, wherein the complementary RNA transcripts comprise protein-encoding open reading frames (ORFs) or non-protein-coding genes.

10. The recombinant poxvirus of claim 9, wherein the complementary portions of the RNA transcripts comprise 50 or more nucleotides.

11. The recombinant poxvirus of claim 10, wherein the complementary portions of the RNA transcripts comprise between 50 and 700 nucleotides.

12. The recombinant poxvirus of claim 10, wherein the complementary portions of the RNA transcripts comprise more than 700 nucleotides.

13. The recombinant poxvirus of claim 10, wherein the heterologous nucleic acids encoding completely or partially complementary RNA transcripts are identical within the complementary region, or have a similarity of more than 99%, of more than 95%, of more than 90%, of more than 80%, or of more than 70% wihin the complementary region.

14. The recombinant poxvirus of claim 13, wherein the two identical or highly similar sequences encoding partially or completely complementary transcripts are separated by one or more essential viral genes.

15. The recombinant poxvirus of claim 14, wherein a sense messenger RNA (mRNA) is transcribed from one of the two identical or highly similar sequences and an anti-sense mRNA is transcribed from the other identical or highly similar sequence.

16. The recombinant poxvirus of claim 15 wherein sense and antisense RNAs are transcribed from both strands of the same heterologous sequence insert.

17. The recombinant poxvirus of claim 15 wherein sense and antisense RNAs are transcribed from both strands of a native poxvirus sequence, preferably an early gene, more preferably an immediate-early gene.

18. The recombinant poxviruses of any one claims 15 to 17, wherein expression of the sequences encoding complementary RNA transcripts is each directed by poxviral promoters.

19. The recombinant poxvirus of claim 16, wherein the poxviral promoters are early promoters or immediate-early promoters.

20. A method of attenuating standard replication-competent vaccinia virus strains and other species derived from the chordopoxvirinae subfamily by expression of early dsRNA as described in claims 1-19.

21. A method of enhancing innate immune activation comprising administering the recombinant poxvirus of claim 19 to a vertebrate subject; wherein said administration enhances production of type I interferons (type I IFNs), cytokines and chemokines in the subject.

22. The method of claim 21, wherein production of type I IFNs comprises transcription of interferon-beta (IFN-β) -encoding messenger RNA (mRNA), and wherein transcription of IFN-β mRNA increases by at least two-fold.

23. The method of claim 22, wherein transcription of IFN-β-encoding mRNA increases by at least ten-fold.

24. The method of claim 23, wherein transcription of IFN-β-encoding mRNA increases by at least fifty-fold.

25. The method of claim 21, wherein production of type I IFNs comprises secretion of IFN-β protein, and wherein secretion of IFN-β protein increases by at least two-fold.

26. The method of claim 25, wherein secretion of IFN-β protein increases by at least four-fold.

27. The method of claim 26, wherein secretion of IFN-β protein increases by at least ten-fold.

28. The method of claim 21, wherein administration enhances production of the cytokines IFN-α, IFN-γ, IL-6, and IL-18.

29. The method of claim 21, wherein administration enhances production of the chemokines CXCL1, CCL2 and CCL5.

30. The method of claim 21, wherein administration enhances production of vector-specific CD8 T cells, by at least 10%, preferably by 25%, more preferably by more than 50%.

31. The poxvirus of claim 19, which is produced in the chicken fibroblast cell line DF-1 (ATCC®CRL-12203).

innate and adaptive immune response of the host. Among the known poxviral factors counteracting the interferon system are the secreted receptor-like proteins B19 and B8 that bind and neutralize type I and type II interferons, respectively. Other poxviral proteins such as VACV E3, K7, C6, N1, C7, K1, K3 and H1 inhibit the induction of type I interferon or block interferon signaling and effector pathways. The fact that poxviruses encode such a multitude of proteins to counteract the interferon system highlights the importance of this system of innate immune defense for the control and eventual clearance of poxviruses by the infected host organism. Apart from the interferon system, orthopoxviruses encode other immune-modulating proteins interfering with induction or function of cytokines like IL-1β, IL-18, and of chemokines including VACV B16R, WR013, C23L/vCCI. These cytokines are also important in restricting pathogen spread and protecting the host from severe pathogen-induced damage. Poxviral proteins subverting pattern recognition pathways are universally efficient in thwarting induction of IFNs as well as of other cytokines and chemokines.

[010] Accordingly, implementation of the techniques disclosed herein may allow enhanced recognition of poxviruses by cellular dsRNA sensors, as well as an increase to the innate immune activation induced by MVA. Disclosed herein are recombinant poxviruses engineered to produce dsRNA early in infection by inserting two partially identical DNAs at two separate locations in the genome. Those DNAs are operable linked to strong early promoters oriented such that one DNA produces a ‘sense’ transcript and the other produces a complementary ‘antisense’ transcript. The two partially or completely complementary transcripts subsequently anneal to produce dsRNA. Efficient induction of IFN-β, an important marker of innate immune activation, was observed with early dsRNAs over a wide size range, with decreasing efficiencies as the complementary transcripts were shortened down to 50 bp.

SUMMARY

[010a] The invention is defined in the independent claims. Some optional features of the invention are defined in the dependent claims.

[011] There is disclosed a recombinant poxvirus comprising heterologous nucleic acids expressing excess double-stranded RNA (dsRNA) early in infection. Also disclosed is a method of enhancing innate immune activation comprising administering the recombinant poxvirus to a vertebrate subject, wherein said administration enhances production of type I interferons (type I IFNs), cytokines and chemokines in the subject.

[012] The recombinant poxvirus may transcribe sense and antisense RNAs from both strands of a native poxvirus sequence, preferably an early gene, more preferably an immediate-early gene.

[013] The recombinant poxvirus may transcribe heterologous nucleic acid as sense and antisense RNAs from both strands of the heterologous sequence.

[014] Also disclosed is a method of attenuating standard replication-competent vaccinia virus strains and of other species derived from chordopoxvirinae by expression of excess early dsRNA.

[015] The poxvirus may further comprise heterologous sequences encoding one or more costimulatory molecules.

[016] The poxvirus may further comprise heterologous sequences encoding one or more bacterial, viral, fungal, parasite, or tumor antigens.

[017] The poxvirus may be an orthopoxvirus, a parapoxvirus, a yatapoxvirus, an avipoxvirus, a leporipoxvirus, a suipoxvirus, a capripoxvirus, a cervidpoxvirus, or a molluscipoxvirus. The orthopoxvirus may be selected from the group consisting of vaccinia virus, cowpox virus, and monkeypox virus. The vaccinia virus may be a modified vaccinia virus Ankara (MVA), e.g. modified vaccinia virus Ankara Bavarian Nordic (MVA-BN).

[018] The heterologous or endogenous nucleic acids may generate dsRNA comprise sequences encoding partially or completely complementary RNA

transcripts, wherein the complementary portions of RNA transcripts anneal after transcription to form dsRNA.

[019] The heterologous nucleic acids may encode completely or partially complementary RNA transcripts are identical within the complementary region, or have a similarity of more than 99%, of more than 95%, of more than 90%, of more than 80%, or of more than 70% within the complementary region.

[020] Advantages of the techniques disclosed herein will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice.

[021] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

[022] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate one (several) embodiment(s) of the invention and together with the description, serve to explain the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[023] **Figure 1** depicts a schematic representation of CVA mutants overproducing early dsRNA and corresponding control mutants. Boxes represent CVA ORFs in the genomic region between ORFs B14R and B20R and are not drawn to scale. The box representing the CVA version of the B15 ORF is shown in black, whereas the MVA version of B15R is indicated by a diamond pattern. A hatched box represents the B19R ORF. All other ORFs are shown as grey boxes. The bacterial selection markers (neo^r and zeo^r) replacing CVA ORFs in the various deletion mutants are shown as smaller boxes with an indication of the specific marker.

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[050] As used herein, the singular forms “a”, “an”, and “the” include plural references unless the context clearly indicates otherwise. Thus, for example, reference to “an epitope” includes reference to one or more epitopes and reference to “the method” includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods provided herein.

[051] Unless otherwise indicated, the term “at least” preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments provided herein. Such equivalents are encompassed by the present invention.

[052] Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising” mean “includes”, and therefore include a stated integer or step or group of integers or steps and do exclude any other integer or step or group of integers or steps. When used herein the term “comprising” can be substituted with the term “containing”, “including” or “having”. Any of the aforementioned terms (comprising, containing, including, having), whenever used herein in the context of an aspect or embodiment of the present disclosure can be substituted with the term “consisting of”.

[053] When used herein, the term “consisting of” excludes any element, step, or ingredient not specified in the claim. When used herein, “consisting essentially of” excludes any materials or steps “which would affect the basic and novel characteristics” of the product or

[067] **CD4**. Cluster of differentiation factor 4, a T-cell surface protein that mediates interaction with the MHC Class II molecule. Cells that express CD4, referred to as "CD4+" cells, are often helper T (*e.g.*, "T_H", "T_H1" or "T_H2") cells.

[068] **CD8**. Cluster of differentiation factor 8, a T-cell surface protein that mediates interaction with the MHC Class I molecule. Cells that express CD8, referred to as "CD8+" cells, are often cytotoxic T ("CTL") cells.

[069] **Costimulatory molecule**. T-cell activation typically requires binding of the T-cell receptor ("TCR") with a peptide-MHC complex as well as a second signal delivered via the interaction of a costimulatory molecule with its ligand. Costimulatory molecules are molecules that, when bound to their ligand, deliver the second signal required for T-cell activation. The most well-known costimulatory molecule on the T-cell is CD28, which binds to either B7-1 or B7-2. Other costimulatory molecules that can also provide the second signal necessary for activation of T-cells include intracellular adhesion molecule-1 ("ICAM-1"), intracellular adhesion molecule-2 ("ICAM-2"), leukocyte function associated antigen-1 ("LFA-1"), leukocyte function associated antigen-2 ("LFA-2"), and leukocyte function associated antigen-3 ("LFA-3"). The combination of B7-1, ICAM-1, and LFA-3 is referred to as "TRICOM", for "TRIad of COstimulatory Molecules".

[070] **Dendritic cell (DC)**. Dendritic cells are the main antigen presenting cells ("APCs") involved in primary immune responses. Dendritic cells include plasmacytoid dendritic cells and myeloid dendritic cells. Their major function is to obtain antigen in tissues, migrate to lymphoid organs and present the antigen in order to activate T-cells. Immature dendritic cells originate in the bone marrow and reside in the periphery as immature cells.

[071] **Double-stranded RNA (dsRNA).** Recombinant poxvirus virus comprising heterologous or native nucleic acids expressing an increased number or amount of dsRNA (e.g. expressing excess dsRNA) may trigger the release of cytokines, chemokines, effector molecules and/or increased expression of

costimulatory molecules or activates one or more pattern recognition receptor(s) (PRRs) and/or activates cells (e.g dendritic cells, macrophages, B cells or other types of immune cells) and thus preferably triggers or induces an enhanced innate immune response. Excess dsRNA can be determined by any method suitable, preferably by using a method to determine enhanced innate immune response or any method as described in the examples preferably the method according to Example 16. Preferably, excess dsRNA can be defined as the amount of dsRNA early in infection that generates an enhanced innate immune response when using the recombinant poxvirus vector as disclosed herein. More preferably, excess dsRNA can be defined as the amount of dsRNA transcribed during the early phase of virus infection with the recombinant poxvirus comprising heterologous nucleic acids expressing or generating dsRNA compared to the control. Excess dsRNA can be generated by driving transcription of sense and antisense RNA with identical or overlapping sequence stretches preferably of at least 100 bp in length by using poxviral promoters with early activity (e,g, immediate early, early, or early/late poxviral promoters). Another method of determining excess dsRNA is to determine enhanced activation of one or more PRR(s) e.g. PKR as determined by increased phosphorylation of a PKR substrate (e.g. eIF2α at position Serine-51) as shown in Example 7. Preferably, increased phosphorylation of a PKR substrate (e.g. eIF2α at position Serine-51) increases by at least 2-fold, at least 4-fold, at least 6-fold, at least 8-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 100-fold or more as compared to the control.

[072] **Early in infection**. Gene expression of poxviruses is regulated in a cascade fashion. Early transcription of viral genes is driven by the viral transcriptional machinery carried with the viral particle into the newly infected cell and is under control of early viral promoters that are recognized by the early viral transcription complex. It is well known that once the virion and host membranes have fused and the virus core has been released into the cytoplasm, the endogenous RNA polymerase and encapsidated transcription factors that comprise the viral

CLAIMS:

1. A recombinant poxvirus comprising heterologous nucleic acids expressing excess double-stranded RNA (dsRNA) early in infection, wherein the heterologous nucleic acids generating dsRNA comprise sequences encoding partially or completely complementary RNA transcripts, wherein the complementary portions of RNA transcripts anneal after transcription to form dsRNA and wherein the complementary portions of the RNA transcripts overlap by more than 100 nucleotides.

2. The recombinant poxvirus of claim 1, further comprising heterologous sequences encoding one or more costimulatory molecules.

3. The recombinant poxvirus of claim 2, wherein the one or more costimulatory molecules is TRICOM (B7-1, ICAM-1, and LFA-3).

4. The recombinant poxvirus of any one of claims 1 to 3, further comprising heterologous sequences encoding one or more bacterial, viral, fungal, parasite, or tumor antigens.

5. The recombinant poxvirus of any one of claims 1 to 4, wherein the poxvirus is an orthopoxvirus, a parapoxvirus, a yatapoxvirus, an avipoxvirus, a leporipoxvirus, a suipoxvirus, a capripoxvirus, a cervidpoxvirus, or a molluscipoxvirus..

6. The recombinant poxvirus of claim 5, wherein the orthopoxvirus is selected from the group consisting of vaccinia virus, cowpox virus, and monkeypox virus.

7. The recombinant poxvirus of claim 6, wherein the vaccinia virus is selected from the group consisting of vaccinia virus-Western Reserve, vaccinia virus-Copenhagen, Dryvax, vaccinia virus Lister, vaccinia virus Acambis 2000 and 3000, rabbitpox virus, buffalopox virus, modified vaccinia virus Ankara (MVA), and modified vaccinia virus Ankara-Bavarian Nordic (MVA-BN).

8. The recombinant poxvirus of claim 6, wherein the complementary RNA transcripts comprise protein-encoding open reading frames (ORFs) or non-protein-coding genes.

9. The recombinant poxvirus of any one of claims 1 to 8, wherein the complementary portions of the RNA transcripts overlap by between 100 and 1000 nucleotides, preferably between 300 and 1000 nucleotides.

10. The recombinant poxvirus of claims 1 to 8, wherein the complementary portions of the RNA transcripts overlap by more than 700 nucleotides.

11. The recombinant poxvirus of claim 8, wherein the heterologous nucleic acids encoding completely or partially complementary RNA transcripts are identical within the complementary region, or have a similarity of more than 95%, within the complementary region.

12. The recombinant poxvirus of claim 11, wherein the two identical or highly similar sequences encoding partially or completely complementary transcripts are separated by one or more essential viral genes.

13. The recombinant poxvirus of claim 12, wherein a sense messenger RNA (mRNA) is transcribed from one of the two identical or highly similar sequences and an anti-sense mRNA is transcribed from the other identical or highly similar sequence.

14. The recombinant poxvirus of claim 13, wherein sense and antisense RNAs are transcribed from both strands of the same heterologous sequence insert.

15. The recombinant poxvirus of claim 13, wherein sense and antisense RNAs are transcribed from both strands of a native poxvirus sequence, preferably an early gene, more preferably an immediate-early gene.

16. The recombinant poxviruses of any one of claims 13 to 15, wherein expression of the sequences encoding complementary RNA transcripts are each directed by poxviral promoters.

17. The recombinant poxvirus of claim 16, wherein the poxviral promoters are early promoters or immediate-early promoters.

18. An immunogenic or pharmaceutical composition comprising the recombinant poxvirus of claims 1 to 17 and a pharmaceutically acceptable carrier or excipient.

19. An in vitro method of attenuating standard replication-competent vaccinia virus strains and other species derived from the chordopoxvirinae subfamily by expression of early dsRNA as described in claims 1-17.

20. Use of the recombinant poxvirus of claims 1 to 17 for manufacturing of a pharmaceutical composition for enhancing innate immune activation in a vertebrate subject.

21. The use of the recombinant poxvirus of claim 20 for enhancing innate immune activation in a vertebrate subject; wherein said pharmaceutical composition enhances production of type I interferons (type I IFNs), cytokines and chemokines in the subject.

22. The use of the recombinant MVA of claim 21, wherein production of type I IFNs comprises transcription of interferon-beta (IFN-β) -encoding messenger RNA (mRNA), and wherein transcription of IFN-β mRNA increases by at least two-fold.

23. The use of the recombinant MVA of claim 22, wherein transcription of IFN-β-encoding mRNA increases by at least ten-fold.

24. The use of the recombinant MVA of claim 23, wherein transcription of IFN-β-encoding mRNA increases by at least fifty-fold.

25. The use of the recombinant poxvirus of claim 21, wherein production of type I IFNs comprises secretion of IFN-β protein, and wherein secretion of IFN-β protein increases by at least two-fold.

26. The use of the recombinant poxvirus of claim 25, wherein secretion of IFN-β protein increases by at least four-fold.

27. The use of the recombinant poxvirus of claim 26, wherein secretion of IFN-β protein increases by at least ten-fold.

28. The use of the recombinant poxvirus of claim 21, wherein administration of the pharmaceutical composition enhances production of the cytokines IFN-α, IFN-γ, IL-6, and IL-18.

29. The use of the recombinant poxvirus of claim 21, wherein administration of the pharmaceutical composition enhances production of the chemokines CXCL1, CCL2 and CCL5.

30. The use of the recombinant poxvirus of claim 21, wherein administration of the pharmaceutical composition enhances production of vector-specific CD8 T cells, by at least 10%, preferably by 25%, more preferably by more than 50%.

31. The recombinant poxvirus of any one of claims 1 to 17, wherein the recombinant poxvirus used for generating the recombinant poxvirus is MVA-BN or a derivative thereof.