The rdCMV described herein comprises a nucleic acid encoding one or more fusion proteins that comprise an essential protein fused to a destabilizing protein. In the absence of a stabilizing agent, the fusion protein is degraded. Thus, the rdCMV can be grown in tissue culture under conditions that allow for replication (i.e., in the presence of the stabilizing agent) but replication is reduced, and preferably prevented, when administered to a patient (in the absence of the stabilizing agent).

In a first aspect, there is provided a conditional replication defective cytomegalovirus (CMV) comprising: (a) a pentameric gH complex comprising of UL128, UL130, UL131, gH and gL; and (b) a nucleic acid encoding a first fusion protein between an essential protein IE1/2 and a destabilizing protein and a second fusion protein between UL51 and the destabilizing protein, wherein the destabilizing protein is either FK506-binding protein (FKBP) or a FKBP derivative comprising amino acid substitutions F36V and L106P, wherein the wild type IE1/2 and UL51 are no longer present and wherein the CMV is an attenuated strain that has restored gH complex expression due to a repair of a mutation in the UL131 gene, ~~wherein the essential protein is selected from the group consisting of IE1/2, UL51, UL52, UL79 and UL84~~.

One embodiment of the present disclosure is a conditional replication defective CMV. The rdCMV comprises a nucleic acid encoding one or more fusion proteins that comprise an essential protein fused to a destabilizing protein. The nucleic acids encoding the wild type essential protein are no longer present in the rdCMV and thus the fusion protein is required for viral replication. In preferred embodiments, the essential proteins are selected from the group consisting of IE1/2, UL51, UL52, UL79 and UL84 and the destabilizing protein is FKBP or a derivative thereof.

In a second aspect, there is provided a composition comprising the conditional replication defective CMV as defined above and a pharmaceutically acceptable carrier.

Another embodiment of the present disclosure is a composition comprising an isolated rdCMV and a pharmaceutically acceptable carrier. The composition can further comprise an adjuvant including, but no limited to ISCOMATRIX® adjuvant and aluminium phosphate adjuvant.

In a third aspect, there is provided a composition as defined above in an immunological effective amount for use in inducing an immune response against CMV in a patient.

In a fourth aspect, there is provided use of a conditional replication defective CMV as defined above in the manufacture of a medicament for inducing a protective immune response in a patient against CMV infection.

Another embodiment of the present disclosure is use of the rdCMV composition to induce an immune response against CMV in a patient. Patients can be treated prophylactically or therapeutically by administration of the rdCMV of the present disclosure. Prophylactic treatment provides sufficient protective immunity to reduce the likelihood or severity of a CMV infection, including primary infections, recurrent infections (i.e., those resulting from reactivation of latent CMV) and super-infections (i.e., those resulting from an infection with a different stain of CMV than previously experienced by the patient). In specific embodiments, females of childbearing age, especially early adolescent females, are vaccinated to decrease the likelihood of CMV infection (either primary, recurrent or super) during pregnancy and thus decrease the likelihood of transmission of CMV to the fetus. Therapeutic treatment can be performed to reduce the length/severity of a current CMV infection.

In a fifth aspect, there is provided a method of making the conditional replication defective CMV as defined above comprising propagating the recombinant CMV in epithelial cells in the presence of Shield-1.

Another embodiment of the present disclosure is methods of making the rdCMV of the disclosure comprising propagating the rdCMV on epithelial cells, such as ARPE-19 cells (ATCC Accession No. CRL-2302), in the presence of Shield-1. In some embodiments, the

rdCMV is propagated on epithelial cells on microcarriers or other high density cell culture systems.

In a sixth aspect, there is provided a composition comprising the conditional replication defective CMV as defined above in a buffer at a pH between 5-7~~, wherein the buffer comprises~~ comprising:

(a) between 15-35 mM Histidine; and

(b) between 100-200 mM NaCl.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1B shows a schematic diagram of the construction of a strain of CMV with restored expression of the pentameric gH complex. **(A)** Strategy for generation of self-excisable Bacterial Artificial Chromosome (BAC) to manipulate AD169 viral genome. **(B)** Repair of the frame shift mutation in UL131 to restore its expression. **(C)** Replacement of GFP with a cre recombinase gene to create a self excisable CMV BAC.

FIGS. 2A-2D show the effect of conventional inactivation methods on gH complex immunogenicity. γ-irradiation **(A, B)** and ß-propiolactonE (BPL) **(C, D)** were used to inactivate gH complex expressing CMV. Inactivation kinetics were determined by plaque assay **(A, C)** while immunogenicity was determined by evaluating sera from mice administered the CMV for neutralizing activity against viral epithelial cell entry **(B, D)**.

FIG. 3 shows the Shield 1 concentration dependent progeny virus production of gH complex expressing CMV with various essential proteins fused to a FKBP derivative. ARPE-19 cells were infected with the rdCMV viruses at a multiplicity of 0.01 PFU/cell for 1 h, washed twice with fresh medium, and incubated in the growth medium containing 0, 0.05, 0.1 0.5 or 2 μM of Shield-1. Seven days post infection, the cell free virus was collected, and virus titers were determined by TCID50 assay on ARPE-19 cells in the presence of 2 μM of Shield 1.

FIGS. 4A-4D show growth kinetics of rdCMV in ARPE-19 cells. Cells were infected with viruses containing **(A)** IE1/2, **(B)** UL51, **(C)** IE1/2-UL51 fusion proteins or the **(D)** parental

WHAT IS CLAIMED IS:

1. A conditional replication defective cytomegalovirus (CMV) comprising:

(a) a pentameric gH complex comprising of UL128, UL130, UL131, gH and gL; and

(b) a nucleic acid encoding a first fusion protein between an essential protein IE1/2 and a destabilizing protein and a second fusion protein between UL51 and the destabilizing protein, ~~wherein the essential protein is selected from the group consisting of IE1/2, UL51, UL52, UL79 and UL84~~ wherein the destabilizing protein is either FK506-binding protein (FKBP) or a FKBP derivative comprising amino acid substitutions F36V and L106P, wherein the wild type IE1/2 and UL51 are no longer present and wherein the CMV is an attenuated strain that has restored gH complex expression due to a repair of a mutation in the UL131 gene.

~~2. The conditional replication defective CMV of claim 1, wherein the destabilizing protein is either FKBP or an FKBP derivative, wherein the FKBP derivative is FKBP comprising one or more amino acid substitutions selected from the group consisting of: F15S, V24A, H25R, F36V, E60G, M66T, R71G, D100G, D100N, E102G, K105I and L106P.~~

~~3~~2. The conditional replication defective CMV of claim ~~2~~1, wherein the ~~FKBP derivative~~destablisizing protein is a FKBP derivative comprising amino acid substitutions F36V and L106P.

~~4. The conditional replication defective CMV of any of claims 1-3, wherein the essential protein is IE1/2 or UL51.~~

~~5. The conditional replication defective CMV of any of claims 1-3, wherein the CMV comprises a nucleic acid encoding at least two fusion proteins, wherein the essential proteins in each of the fusion proteins are different.~~

~~6. The conditional replication defective CMV of claim 5, wherein one of the fusion proteins comprises IE1/2 or UL51, or wherein the CMV comprises a first fusion protein comprising IE1/2 and a second fusion protein comprising UL51.~~

~~7~~3. The conditional replication defective CMV of claim ~~6~~1 or 2, wherein

(a) the first fusion protein is SEQ ID NO:1 or an amino acid that is at least 95% identical to SEQ ID NO:1; and

(b) the second fusion protein is SEQ ID NO:3 or an amino acid that is at least 95% identical to SEQ ID NO:3.

~~8~~4. The conditional replication defective CMV of claim ~~7~~3, wherein

(a) the first fusion protein is encoded by SEQ ID NO:2 or a nucleic acid that is at least 95% identical to SEQ ID NO:2; and

(b) the second fusion protein is encoded by SEQ ID NO:4 or a nucleic acid that is at least 95% identical to SEQ ID NO:4.

5. The conditional replication defective CMV of any one of claims 1-4, wherein the CMV is AD169 that has restored gH complex expression due to a repair of a mutation in the UL131 gene.

~~9~~6. A composition comprising the conditional replication defective CMV of any of claims 1-~~8~~5 and a pharmaceutically acceptable carrier.

~~10~~7. The composition of claim ~~9~~6 further comprising an adjuvant.

~~11~~8. The composition of ~~any of~~ claims ~~9-10~~6 or 7 in an immunological effective amount for use in inducing an immune response against CMV in a patient.

~~12~~9. The composition of claim ~~18~~8, wherein the immune response is protective immune response in a patient against CMV infection.

~~13~~10. The composition of claim ~~11-~~8 or ~~12~~9, wherein the patient ~~is a human with~~has weakened immunity.

~~14~~11. The composition of claim ~~11-~~8 or ~~12~~9, wherein the patient is a human female of childbearing age.

~~15~~12. Use of a conditional replication defective CMV of any of claims 1-~~8-~~5 in the manufacture of a medicament for inducing a protective immune response in a patient against CMV infection.

~~16~~13. The conditional replication defective CMV of any of claims 1-~~8-~~5 for use in a medicine to treat CMV infection in a patient.

~~17~~14. A method of making the conditional replication defective CMV of any of claims ~~21-8-~~5 comprising propagating the recombinant CMV in epithelial cells in the presence of Shield-1.

~~18~~15. The method of claim ~~17~~14, wherein the epithelial cells are human pigmented retinal epithelial cells.

~~19~~16. The method of claim ~~18~~15, wherein the human retinal pigmented epithelial cells are ARPE-19 cells deposited with the American Type Culture Collection (ATCC) as Accession No. CRL-2302.

~~20~~17. The method of claim ~~17~~14, wherein the Shield-1 is present at a concentration of at least 0.5 μM.

~~21~~18. A composition comprising the conditional replication defective CMV of any of claims 1-~~8~~ 5 in a buffer at a pH between 5-7~~, wherein the buffer comprises~~ comprising:

(a) between 15-35 mM Histidine; and

(b) between 100-200 mM NaCl.

~~22~~19. The composition of claim ~~21~~ 18 wherein the buffer comprises 25 mM Histidine with 150 mM NaCl at a pH of 6.

~~23~~20. The composition of claim ~~22~~ 19 further comprising 9% w/v sucrose.