variety of cell types, laboratory strains AD169 (Elek & Stern, 1974, Lancet 1:1) and Towne (Plotkin et al., 1975, Infect. Immun. 12:521) replicate almost exclusively in fibroblasts (Hahn et al., 2004, J. Virol. 78:10023). The restriction in tropism, which results from serial passages and eventual adaptation of the virus in fibroblasts, is stipulated a marker of attenuation (Gerna et al., 2005, J. Gen. Virol. 86:275; Gerna et al, 2002, J. Gen Virol. 83:1993; Gerna et al, 2003, J. Gen Virol. 84:1431; Dargan et al, 2010, J. Gen Virol. 91:1535). Mutations causing the loss of epithelial cell, endothelial cell, leukocyte, and dendritic cell tropism in human CMV laboratory strains have been mapped to three open reading frames (ORFs): UL128, UL130, and UL131 (Hahn et al., 2004, J. Virol. 78:10023; Wang and Shenk, 2005 J. Virol. 79:10330; Wang and Shenk, 2005 Proc Natl Acad Sci USA. 102:18153). Biochemical and reconstitution studies show that UL128, UL130 and UL131 assemble onto a gH/gL scaffold to form a pentameric gH complex (Wang and Shenk, 2005 Proc Natl Acad Sci USA. 102:1815; Ryckman et al, 2008 J. Virol. 82:60). Restoration of this complex in virions restores the viral epithelial tropism in the laboratory strains (Wang and Shenk, 2005 J. Virol. 79:10330).

Loss of endothelial and epithelial tropism has been suspected as a deficiency in the previously evaluated as vaccines such as Towne (Gerna et al, 2002, J. Gen Virol. 83:1993; Gerna et al, 2003, J. Gen Virol. 84:1431). Neutralizing antibodies in sera from human subjects of natural CMV infection have more than 15-fold higher activity against viral epithelial entry than against fibroblast entry (Cui et al, 2008 Vaccine 26:5760). Humans with primary infection rapidly develop neutralizing antibodies to viral endothelial and epithelial entry but only slowly develop neutralizing antibodies to viral fibroblast entry (Gerna et al, 2008 J. Gen. Virol. 89:853). Furthermore, neutralizing activity against viral epithelial and endothelial entry is absent in the immune sera from human subjects who received Towne vaccine (Cui et al, 2008 Vaccine 26:5760). More recently, a panel of human monoclonal antibodies from four donors with HCMV infection was described, and the more potent neutralizing clones from the panel recognized the antigens of the pentameric gH complex (Macagno et al, 2010 J. Virol. 84:1005).

SUMMARY OF THE INVENTION

The present ~~invention~~ disclosure is directed to conditional replication defective CMV (rdCMV) and the use of rdCMV in compositions and methods of treating and/or decreasing the likelihood of an infection by CMV or pathology associated with such an infection in a patient.

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 fusion protein between an essential protein and a destabilizing protein, wherein the essential protein is selected from the group consisting of IE1/2, UL51, UL52, UL79 and UL84.

One embodiment of the present disclosure ~~invention~~ 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 ~~invention~~ 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 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 ~~invention~~ 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~~invention~~. 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 ~~invention~~ is methods of making the rdCMV of the disclosure ~~invention~~ 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:

(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

beMAD virus at multiplicity of 0.01 PFU/cell. After one hour, the cells were washed twice with fresh medium, and incubated in the absence (open circle) or presence (closed circle) of 2 μM of Shield-1. Cell-free virus was collected at the indicated time points after infection, and infectious virus was quantified by TCID50 assay on ARPE-19 cells in the medium containing 2 μM of Shield-1.

11. ~~A method of inducing an immune response against CMV in a patient comprising administering to the patient an immunologically effective amount of the~~ The composition of any of claims 9-10 for use in inducing an immune response against CMV in a patient.

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

13. The composition ~~method~~ of claim 11 or 12, wherein the patient is a human with weakened immunity.

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

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

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