CYTOMEGALOVIRUS VECTORS ELICITING T CELLS RESTRICTED BY MAJOR HISTOCOMPATIBILITY COMPLEX E MOLECULES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority from U.S. Provisional Application No. 62/409,840, filed on October 18, 2016, the disclosure of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present invention relates to the use of cytomegalovirus (CMV) vectors in immunization, and more specifically, the generation of T cell responses characterized by MHC-E restriction. Particular embodiments relate to the generation of CD8+ T cells that are restricted by MHC-E.

ACKNOWLEDGEMENT OF GOVERNMENT SUPPORT

[0003] This disclosure was created with the support of the United States government under the terms of grant numbers P01 AI094417 and R01 AI117802, awarded by the National Institutes of Health. The United States government has certain rights in this disclosure.

REFERENCE TO A SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

[0004] The content of the electronically submitted sequence listing (Name: 3919.014PC01_ST25; Size: 1,063 bytes; and Date of Creation: October 18, 2017) is herein incorporated by reference in its entirety.

SUMMARY

[0004a] In one aspect, the present disclosure refers to a cytomegalovirus (CMV) vector comprising (a) a first nucleic acid sequence that encodes at least one heterologous antigen; and (b) a second nucleic acid sequence comprising a first microRNA recognition element (MRE) operably linked to a CMV gene that is essential or augmenting for CMV growth, wherein the MRE silences expression of the CMV gene that is essential or augmenting for CMV growth in

the presence of miR-126-3p; wherein the vector does not express an active UL128 protein or homolog thereof; does not express an active UL130 protein or homolog thereof; does not express an active UL146 or homolog thereof; and does not express an active UL147 protein or homolog thereof. In one example, there is disclosed a cytomegalovirus (CMV) vector comprising: (a) a first nucleic acid sequence that encodes at least one heterologous antigen; and (b) a second nucleic acid sequence comprising a first microRNA recognition element (MRE) operably linked to a CMV gene that is essential or augmenting for CMV growth, wherein the MRE silences expression in the presence of a microRNA that is expressed by a cell of endothelial lineage; wherein the vector does not express an active UL128 protein or ortholog thereof; does not express an active UL130 protein or ortholog thereof; does not express an active UL146 or ortholog thereof; and does not express an active UL147 protein or ortholog thereof.

[0004b] In another aspect, the present disclosure refers to use of the CMV or HCMV vector as disclosed herein in the manufacture of a medicament for treating cancer.

[0004c] In yet another aspect, the present disclosure refers to use of the CMV or HCMV vector as disclosed herein in the manufacture of a medicament for treating a pathogenic infection.

[0004d] In yet another aspect, the present disclosure refers to use of the CMV or HCMV vector as disclosed herein in the manufacture of a medicament for treating an autoimmune disease or disorder.

[0004e] In yet another aspect, the present disclosure refers to an *in vitro* method of generating CD8+ T cells that recognize MHC-E/peptide complexes, the method comprising (1) a set of CD8+ T cells that recognize MHC-E/peptide complexes to be isolated from a subject, where the subject had been administered the CMV or HCMV vector of as disclosed herein; (2) identifying a first CD8+ TCR from the set of CD8+ T cells, wherein the first CD8+ TCR recognizes a MHC-E/heterologous antigen-derived peptide complex; (3) one or more CD8+ T cells to be isolated from the subject; and (4) transfecting the one or more CD8+ cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the second CD8+ TCR, wherein the second CD8+ TCR comprises CDR3α and CDR3β of the first CD8+ TCR, thereby generating one or more transfected CD8+ T cells that recognize a MHC-E/heterologous antigen-derived peptide complex. In another example, there is disclosed a method of generating CD8+ T cells that recognize MHC-E-peptide complexes, the method comprising:

(1) the CMV or HCMV vector as disclosed herein to be administered to a subject in an amount effective to generate a set of CD8+ T cells that recognize MHC-E/peptide complexes; (2) identifying a first CD8+ TCR from the set of CD8+ T cells, wherein the first CD8+ TCR recognizes a MHC-E/heterologous antigen-derived peptide complex; (3) one or more CD8+ T cells to be isolated from the subject; and (4) transfecting the one or more CD8+ cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the second CD8+ TCR, wherein the second CD8+ TCR comprises CDR3α and CDR3β of the first CD8+ TCR, thereby generating one or more transfected CD8+ T cells that recognize a MHC-E/heterologous antigen-derived peptide complex.

[0004f] In yet another aspect, the present disclosure refers to an *in vitro* method of generating CD8+ T cells that recognize MHC-E/peptide complexes, the method comprising (1) a set of CD8+ T cells that recognize MHC-E/peptide complexes to be isolated from a subject, where the subject had been administered the CMV vector of as disclosed herein; (2) identifying a first CD8+ TCR from the set of CD8+ T cells, wherein the first CD8+ TCR recognizes a MHC-E/heterologous antigen-derived peptide complex; (3) one or more CD8+T cells to be isolated from a second subject; and (4) transfecting the one or more CD8+T cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the second CD8+ TCR, wherein the second CD8+ TCR comprises CDR3α and CDR3β of the first CD8+ TCR, thereby generating one or more transfected CD8+ T cells that recognize a MHC-E/heterologous antigen-derived peptide complex. In another example, there is described a method of generating CD8+ T cells that recognize MHC-E-peptide complexes, the method comprising: (1) the CMV vector as disclosed herein to be administered to a first subject in an amount effective to generate a set of CD8+ T cells that recognize MHC-E/peptide complexes; (2) identifying a first CD8+ TCR from the set of CD8+ T cells, wherein the first CD8+ TCR recognizes a MHC-E/heterologous antigen-derived peptide complex; (3) one or more CD8+T cells to be isolated from a second subject; and (4) transfecting the one or more CD8+T cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the second CD8+ TCR, wherein the second CD8+ TCR comprises CDR3α and CDR3β of the first

CD8+ TCR, thereby generating one or more transfected CD8+ T cells that recognize a MHC-E/heterologous antigen-derived peptide complex.

[0005] Genetically modified CMV vaccine vectors are disclosed herein. In examples of the genetically modified CMV vaccine vectors, the genetic modifications described herein change the epitope targeting and Major Histocompatibility Complex (MHC) restriction of CD8+ T cell responses elicited by the CMV vaccine vectors, including the ability to elicit CD8+ T cell responses that recognize unique epitopes restricted by cell surface MHC-II and MHC-E proteins.

Claims

1. A cytomegalovirus (CMV) vector comprising:

(a) a first nucleic acid sequence that encodes at least one heterologous antigen; and

(b) a second nucleic acid sequence comprising a first microRNA recognition element (MRE) operably linked to a CMV gene that is essential or augmenting for CMV growth, wherein the MRE silences expression of the CMV gene that is essential or augmenting for CMV growth in the presence of miR-126-3p;

wherein the vector does not express an active UL128 protein or homolog thereof; does not express an active UL130 protein or homolog thereof; does not express an active UL146 or homolog thereof; and does not express an active UL147 protein or homolog thereof.

2. The CMV vector of claim 1, wherein the MRE silences the expression of UL122 (IE2) and UL79 in the presence of miR-126-3p.

3. The CMV vector of claim 1, further comprising:

(c) a third nucleic acid sequence comprising a second MRE operably linked to a CMV gene that is essential or augmenting for CMV growth, wherein the MRE silences expression in the presence of a microRNA that is expressed by a cell of myeloid lineage.

4. The CMV vector of claim 2 or claim 3, wherein the second nucleic acid sequence comprises an MRE that silences expression in the presence of one or more of miR-126-3p, miR-130a, miR-210, miR-221/222, miR-378, miR-296, and miR-328.

5. The CMV vector of claim 4, wherein the second nucleic acid sequence comprises an MRE that silences expression in the presence of miR-126-3p.

6. The CMV vector of claim 4, wherein the MRE silences the expression of UL122 (IE2) and UL79 in the presence of miR-126-3p.

7. The CMV vector of claims 3-6, wherein the third nucleic acid sequence comprises an MRE that silences expression in the presence of one or more of miR-142-3p, miR-223, miR-27a, miR-652, miR-155, miR146a, miR-132, miR-21, and miR-125.

8. The CMV vector of claim 7, wherein the third nucleic acid sequence comprises an MRE that silences expression in the presence of miR-142-3p.

9. The CMV vector of any one of claims 1-8, wherein the at least one heterologous antigen comprises a pathogen-specific antigen, a tumor antigen, a tissue -specific antigen, or a host self -antigen.

10. The CMV vector of claim 9, wherein the host self-antigen is an antigen derived from the variable region of a T cell receptor (TCR) or an antigen derived from the variable region of a B cell receptor.

11. The CMV vector of claim 9, wherein the pathogen-specific antigen is derived from a pathogen selected from the group consisting of: human immunodeficiency virus, simian immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, Plasmodium parasites, and *Mycobacterium tuberculosis*.

12. The CMV vector of claim 9, wherein the tumor antigen is related to a cancer selected from the group consisting of: acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, pancreatic cancer, colon cancer, renal cell carcinoma (RCC), and germ cell tumors.

13. The CMV vector of claims 1-12, wherein the CMV vector does not express an active UL82 (pp71) protein, or a homolog thereof.

14. The CMV vector of any one of claims 1-13, wherein the CMV vector does not express an active US11 protein, or a homolog thereof.

15. The CMV vector of any one of claims 1-14, wherein the CMV vector does not express an active UL82 (pp71) protein or an active US11 protein, or homologs thereof.

16. The CMV vector of any one of claims 1-15, wherein the CMV vector is a human CMV vector (HCMV), a cynomolgus CMV (CyCMV) vector, or a rhesus CMV (RhCMV) vector.

17. Use of the CMV or HCMV vector of any one of claims 1 to 9 and 13-16 in the manufacture of a medicament for treating cancer.

18. Use of the CMV or HCMV vector of any one of claims 1-9, 11, and 13-16 in the manufacture of a medicament for treating a pathogenic infection.

19. Use of the CMV or HCMV vector of any one of claims 1-10 and 13-16 in the manufacture of a medicament for treating an autoimmune disease or disorder.

20. An in vitro method of generating CD8+ T cells that recognize MHC-E/peptide complexes, the method comprising:

(1) a set of CD8+ T cells that recognize MHC-E/peptide complexes to be isolated from a subject, where the subject had been administered the CMV or HCMV vector of claim 1 or claim 2;

(2) identifying a first CD8+ TCR from the set of CD8+ T cells, wherein the first CD8+ TCR recognizes a MHC-E/heterologous antigen-derived peptide complex;

(3) one or more CD8+ T cells to be isolated from the subject; and

(4) transfecting the one or more CD8+ cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the second CD8+ TCR, wherein the second CD8+ TCR comprises CDR3α and CDR3β of the

first CD8+ TCR, thereby generating one or more transfected CD8+ T cells that recognize a MHC-E/heterologous antigen-derived peptide complex.

21. An in vitro method of generating CD8+ T cells that recognize MHC-E/peptide complexes, the method comprising:

(1) a set of CD8+ T cells that recognize MHC-E/peptide complexes to be isolated from a subject, where the subject had been administered the CMV vector of claim 1 or claim 2;

(2) identifying a first CD8+ TCR from the set of CD8+ T cells, wherein the first CD8+ TCR recognizes a MHC-E/heterologous antigen-derived peptide complex;

(3) one or more CD8+T cells to be isolated from a second subject; and

(4) transfecting the one or more CD8+T cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8+ TCR and a promoter operably linked to the nucleic acid sequence encoding the second CD8+ TCR, wherein the second CD8+ TCR comprises CDR3α and CDR3β of the first CD8+ TCR, thereby generating one or more transfected CD8+ T cells that recognize a MHC-E/heterologous antigen-derived peptide complex.