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 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 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 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.

The MHC-II and MHC-E-restricted CD8+ T cell responses elicited by the CMV vaccine vectors described herein are unconventional and observed rarely in natural immune responses to infectious agents. In addition, the breadth and potency of the MHC-II and MHC-E-restricted CD8+ T cell responses elicited by the CMV vaccine vectors described herein are not observed with CMV vaccine vectors that are not genetically modified. In some examples, and without being bound by a particular theory, the inventors believe that MHC-E restricted CD8+ T cells may exploit a lack of pathogen and tumor immune evasion adaptations to MHC-E restricted immune responses, and that the genetically modified CMV vaccine vectors described herein, which may predominantly or exclusively elicit such responses, provide the potential for uniquely potent vaccines against targeted pathogens and tumors. Consistent with this assumption is the finding that only vaccines that elicit MHC-E restricted CD8+ T cells protect against challenge in a non-human primate model for AIDS. In addition, MHC-E has limited polymorphisms such that protective responses that target MHC-E-restricted epitopes are conserved among individuals. Therefore, in some examples, the genetically modified CMV vaccine vectors described herein elicit an MHC-E restricted T cell response that is shared across genetically diverse individuals.

[0006] Disclosed herein are CMV vectors comprising a first nucleic acid sequence that encodes at least one heterologous antigen; and a second nucleic acid sequence comprising a microRNA recognition element (MRE) that silences expression in the presence of a microRNA that is expressed by a cell of endothelial lineage. The MRE is operably linked to a CMV gene that is essential or augmenting for CMV growth. The vectors do not express: an active UL128 protein or ortholog thereof; an active UL130 protein or ortholog thereof; an active UL146 protein or ortholog thereof; or an active UL147 protein or orthologs thereof.

[0007] Also disclosed herein are CMV vectors comprising a first nucleic acid sequence that encodes at least one heterologous antigen; a second nucleic acid sequence comprising a MRE that silences expression in the presence of a microRNA that is expressed by a cell of endothelial lineage; and a third nucleic acid sequence comprising an MRE that silences expression in the presence of a microRNA that is expressed by a cell of myeloid lineage. The MREs are operably linked to a CMV gene that is essential or augmenting for CMV growth. The vectors do not express: an active UL128 protein or ortholog thereof; an active UL130 protein or ortholog thereof; an active UL146 protein or ortholog thereof; or an active UL147 protein or ortholog thereof.

[0008] Also disclosed herein are human cytomegalovirus (HCMV) vectors comprising a nucleic acid sequence that encodes at least one heterologous antigen. The vectors do not express: an active UL128 protein or ortholog thereof; an active UL130 protein or ortholog thereof; an active UL146 protein or ortholog thereof; or an active UL147 protein or ortholog thereof.

[0009] Also disclosed herein are methods of generating an immune response to at least one heterologous antigen in a subject. The methods involve administering to the subject a CMV vector of the type disclosed herein in an amount effective to elicit a CD8+ T cell response to the at least one heterologous antigen. In some examples, at least 10% of the CD8+ T cells elicited by the vector are restricted by MHC-E or an ortholog thereof. In additional examples, fewer than 10% of the CD8+ T cells elicited by the vector are restricted by polymorphic MHC-class Ia or an ortholog thereof. In alternative examples, at least 50% of the CD8+ T cells elicited by the vector are restricted by MHC-class Ia or an ortholog thereof. In yet other examples, at least 10% of the CD8+ T cells elicited by the vector are restricted by MHC-II or an ortholog thereof.

[0010] The heterologous antigen of the CMV vectors disclosed herein may be any heterologous antigen, including a pathogen-specific antigen derived from, for example, human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2) hepatitis B virus, hepatitis C virus, papillomavirus, *Plasmodium* parasites, and *Mycobacterium tuberculosis*. In other examples, the heterologous antigen may be a tumor antigen including, for example, a tumor antigen related to 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, and germ cell tumors. In some examples, the heterologous antigen may be a tissue-specific antigen or a host self-antigen including, for example, an antigen derived from the variable region of a T cell receptor or an antigen derived from the variable region of a B cell receptor.

[0011] Also disclosed herein is a method of generating CD8+ T cells that recognize MHC-E-peptide complexes. This method involves administering to a first subject a CMV vector in an amount effective to generate a set of CD8+ T cells that recognize MHC-E/peptide complexes. The CMV vector comprises a first nucleic acid sequence encoding at least one heterologous antigen and does not express an active UL128 protein or ortholog thereof; an active

UL130 protein or ortholog thereof; an active UL146 protein or ortholog thereof; or an active UL147 protein or ortholog thereof. In some examples, the CMV vector further comprises a second nucleic acid sequence comprising a MRE that silences expression in the presence of a microRNA that is expressed by a cell of endothelial lineage. The heterologous antigen can be any antigen, including a pathogen specific antigen, a tumor antigen, a self-antigen, or a tissue-specific antigen. In some examples, this method may further comprise identifying a first CD8+ T cell receptor from the set of CD8+ T cells, wherein the first CD8+ T cell receptor (TCR) recognizes a MHC-E/heterologous antigen-derived peptide complex. In some examples, the first CD8+ T cell receptor is identified by DNA or RNA sequencing. In some examples, this method may further comprise transfecting one or more T cells isolated from the first subject or a second subject with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8+ T cell receptor, wherein the second CD8+ T cell receptor comprises CDR3α and CDR3β of the first CD8+ T cell receptor, thereby generating one or more transfected CD8+ T cells that recognize a MHC-E/heterologous antigen-derived peptide complex. In some examples, this method may further comprise administering the transfected CD8+ T cells to the first or second subject to treat a disease, such as cancer, a pathogenic infection, or an autoimmune disease or disorder. In some examples, this method may further comprise administering the transfected CD8+ T cells to the first or second subject to induce an autoimmune response to a self-antigen or a tissue-specific antigen.

[0012] Also disclosed herein is a transfected CD8+ T cell that recognizes MHC-E-peptide complexes prepared by a process comprising the steps of: (1) administering to a first subject a CMV vector in an amount effective to generate a set of CD8+ T cells that recognize MHC-E/peptide complexes, (2) identifying a first CD8+ T cell receptor from the set of CD8+ T cells, wherein the first CD8+ T cell receptor recognizes a MHC-E/heterologous antigen-derived complex; (3) isolating one or more CD8+ T cells from the first subject or a second subject; and (4) transfecting the one or more CD8+ T cells isolated from the first or second subject with an expression vector, thereby creating a transfected T cell that recognizes MHC-E-peptide complexes. The CMV vector comprises a first nucleic acid sequence encoding at least one heterologous antigen and does not express an active UL128 protein or ortholog thereof; an active UL130 protein or ortholog thereof; an active UL146 protein or ortholog thereof; or an active UL147 protein or ortholog thereof. In some examples, the CMV vector further comprises a second nucleic acid sequence comprising a MRE that silences expression in the presence of a

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 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.

2. The CMV vector of claim 1, wherein the MRE 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.
3. The CMV vector of claim 2, wherein the MRE silences expression in the presence of miR-126-3p.
4. The CMV vector of claim 3, wherein the MRE silences the expression of UL122 (IE2) and UL79 in the presence of miR-126-3p.
5. 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.

6. The CMV vector of claim 4 or claim 5, 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.
7. The CMV vector of claim 6, wherein the second nucleic acid sequence comprises an MRE that silences expression in the presence of miR-126-3p.
8. The CMV vector of claim 6, wherein the MRE silences the expression of UL122 (IE2) and UL79 in the presence of miR-126-3p.
9. The CMV vector of claims 5-8, 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.
10. The CMV vector of claim 9, wherein the third nucleic acid sequence comprises an MRE that silences expression in the presence of miR-142-3p.
11. The CMV vector of any one of claims 1-10, wherein the at least one heterologous antigen comprises a pathogen□specific antigen, a tumor antigen, a tissue□specific antigen, or a host self□antigen.
12. The CMV vector of claim 11, 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.
13. The CMV vector of claim 11, 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*.
14. The CMV vector of claim 11, 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.

15. The CMV vector of claims 1-14, wherein the CMV vector does not express an active UL82 (pp71) protein, or an ortholog thereof.
16. The CMV vector of any one of claims 1-15, wherein the CMV vector does not express an active US11 protein, or an ortholog thereof.
17. The CMV vector of any one of claims 1-16, wherein the CMV vector does not express an active UL82 (pp71) protein or an active US11 protein, or orthologs thereof.
18. The CMV vector of any one of claims 1-17, wherein the CMV vector is a human CMV vector (HCMV), a cynomolgus CMV (CyCMV) vector, or a rhesus CMV (RhCMV) vector.
19. Use of the CMV or HCMV vector of any one of claims 1 to 11 and 15-18 in the manufacture of a medicament for treating cancer.
20. Use of the CMV or HCMV vector of any one of claims 1-11, 13, and 15-18 in the manufacture of a medicament for treating a pathogenic infection.
21. Use of the CMV or HCMV vector of any one of claims 1-12 and 15-18 in the manufacture of a medicament for treating an autoimmune disease or disorder.
22. A method of generating CD8+ T cells that recognize MHC-E-peptide complexes, the method comprising:

(1) the CMV or HCMV vector of any one of claims 1-4 and 11-18 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.

23. A method of generating CD8+ T cells that recognize MHC-E-peptide complexes, the method comprising:

(1) the CMV vector of any one of claims 1-4 and 11-18 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.