Another unexpected finding was that methods using both EBV lytic antigens and EBV latent antigens in stimulations did not result in a substantial reduction of the frequency of immune cells specific for EBV latent antigens in the expanded population.

The inventors further demonstrate that populations of immune cells comprising immune cells specific for EBV lytic antigens and immune cells specific for EBV latent antigens are more effective for treating EBV-associated disease than populations of immune cells comprising immune cells specific for EBV latent antigens only.

In a first example the present disclosure provides a method for generating or expanding a population of immune cells comprising immune cells specific for an Epstein Barr Virus (EBV) lytic antigen, comprising stimulating immune cells specific for an EBV lytic antigen by contacting peripheral blood mononuclear cells (PBMCs) with: (i) one or more peptides corresponding to all or part of one or more EBV lytic antigens; or (ii) antigen presenting cells (APCs) presenting one or more peptides corresponding to all or part of one or more EBV lytic antigens.

In some examples the method further comprises re-stimulating the immune cells specific for an EBV lytic antigen by contacting them with APCs presenting one or more peptides corresponding to all or part of one or more EBV lytic antigens.

Also provided is a method for generating or expanding a population of immune cells comprising immune cells specific for an Epstein Barr Virus (EBV) lytic antigen and immune cells specific for an EBV latent antigen, comprising stimulating immune cells specific for an EBV lytic antigen and immune cells specific for an EBV latent antigen by contacting peripheral blood mononuclear cells (PBMCs) with: (i) one or more peptides corresponding to all or part of one or more EBV lytic antigens, and one or more peptides corresponding to all or part of one or more EBV latent antigens; or (ii) antigen presenting cells (APCs) presenting one or more peptides corresponding to all or part of one or more EBV lytic antigens, and one or more peptides corresponding to all or part of an EBV latent antigen.

In one aspect, the present disclosure provides a method for generating or expanding a population of immune cells comprising immune cells specific for an Epstein Barr Virus (EBV) lytic antigen and immune cells specific for an EBV latent antigen, comprising stimulating immune cells specific for an EBV lytic antigen and immune cells specific for an EBV latent antigen by contacting peripheral blood mononuclear cells (PBMCs) with: (i) peptides corresponding to EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2; or (ii) antigen presenting cells (APCs) presenting peptides corresponding to EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2.

In some examples the method further comprises re-stimulating the immune cells specific for an EBV lytic antigen and the immune cells specific for an EBV latent antigen by contacting them with APCs presenting one or more peptides corresponding to all or part of one or more EBV lytic antigens, and one or more peptides corresponding to all or part of an EBV latent antigen.

In some examples in accordance with various aspects of the present disclosure the one or more EBV lytic antigens are selected from BZLF1, BRLF1, BMLF1, BMRF1, BXLF1, BALF1, BALF2, BGLF5, BHRF1, BNLF2A, BNLF2B, BHLF1, BLLF2, BKRF4, BMRF2, BALF4, BILF1, BILF2, BNFR1, BVRF2, BALF3, BALF5 and BDLF3. In some examples in accordance with various aspects of the present disclosure the one or more EBV lytic antigens are selected from the group consisting of BZLF1, BRLF1, BMLF1, BMRF1, BXLF1, BALF1, BALF2, BGLF5, BHRF1, BNLF2A, BNLF2B, BHLF1, BLLF2, BKRF4, BMRF2, BALF4, BILF1, BILF2, BNFR1, BVRF2, BALF3, BALF5, BDLF3, and a combination thereof.

Claims:

1. A method for generating or expanding a population of immune cells comprising immune cells specific for an Epstein Barr Virus (EBV) lytic antigen and immune cells specific for an EBV latent antigen, comprising stimulating immune cells specific for an EBV lytic antigen and immune cells specific for an EBV latent antigen by contacting peripheral blood mononuclear cells (PBMCs) with: (i) peptides corresponding to EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2; or (ii) antigen presenting cells (APCs) presenting peptides corresponding to EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2.

2. The method according to claim 1, wherein the method comprises contacting PBMCs with peptides corresponding to EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2.

3. The method according to claim 1 or claim 2, wherein the method comprises contacting PBMCs with EBNA1 pepmix, LMP1 pepmix, LMP2 pepmix, BZLF1 pepmix, BRLF1 pepmix, BMRF1 pepmix, BMLF1 pepmix, BALF2 pepmix, BNLF2A pepmix, BNLF2B pepmix and BMRF2 pepmix.

4. The method according to any one of claims 1 to 3, wherein the PBMCs are PBMCs depleted of CD45RA-positive cells.

5. The method according to any one of claims 1 to 4, wherein stimulating immune cells specific for an EBV lytic antigen and immune cells specific for an EBV latent antigen occurs in the presence of IL-7 and/or IL-15.

6. The method according to any one of claims 1 to 5, wherein the method further comprises re-stimulating the immune cells specific for an EBV lytic antigen and the immune cells specific for an EBV latent antigen by contacting them with APCs presenting peptides corresponding to EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2.

7. The method according to claim 6, wherein re-stimulating the immune cells specific for an EBV lytic antigen and the immune cells specific for an EBV latent antigen occurs in the presence of IL-7 and/or IL-15.

8. The method according to claim 6 or claim 7, wherein re-stimulating the immune cells comprises contacting immune cells specific for EBV antigen(s) with activated T cells (ATCs) presenting peptide(s) corresponding to EBV antigen(s) in the presence of uLCLs.

9. An isolated population of immune cells obtained or obtainable by the method according to any one of claims 1 to 8.

10. A pharmaceutical composition comprising the isolated population of immune cells according to claim 9.

11. Use of the isolated population of immune cells according to claim 9, or the pharmaceutical composition according to claim 10, in the manufacture of a medicament for the treatment or prevention of a disease or disorder associated with EBV infection.

12. The use according to claim 11, wherein the disease or disorder associated with EBV infection is an EBV-associated cancer.

13. The use according to claim 12, wherein the EBV-associated cancer is selected from EBV-positive lymphoma, EBV-positive nasopharyngeal carcinoma, and EBV-positive gastric carcinoma.

14. A method for killing a cell infected with EBV, comprising contacting the cell infected with EBV with the isolated population of immune cells according to claim 9, or the pharmaceutical composition according to claim 10, wherein the method is an *in vitro* method.

15. A method for killing a cancer cell, wherein the cancer cell is infected with EBV, comprising contacting the cancer cell with the isolated population of immune cells according to claim 9, or the pharmaceutical composition according to claim 10, wherein the method is an *in vitro* method.