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(54) Title: HEPATITIS B VIRUS-SPECIFIC T CELL RESPONSES

(57) Abstract: The present disclosure relates to methods to generate an immune response for the treatment or prevention of hepatitis B virus infection. This disclosure also relates to methods to generate MHC-E and/or MHC-II restricted CD8+ T cells for the treatment or prevention of hepatitis B virus infection.

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HEPATITIS B VIRUS-SPECIFIC T CELL RESPONSES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/858,764, filed June 7, 2019, which is hereby incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with government support under grant numbers R01 AI117802, R01 AI129703, R01 AI140888, and P51OD011092 awarded by the National Institutes of Health. The government has certain rights in the invention.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0003] The content of the electronically submitted sequence listing in ASCII text file (Name 4153_011PC01_Seqlisting_ST25; Size: 4,288 bytes; and Date of Creation: June 5, 2020) filed with the application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0004] Chronic hepatitis B virus infection (CHB) is a major global health concern, affecting 247 million individuals worldwide and causing 887,000 deaths annually. While there is an effective prophylactic vaccine available, 10-15% of individuals do not respond adequately to vaccination and are not protected against hepatitis B virus (HBV) infection (Joint Committee on Vaccination and Immunisation. Hepatitis B. In Immunisation Against Infectious Disease, 3rd ed. p. 468.). CHB can lead to progressive liver dysfunction, cirrhosis, and in some cases hepatocellular carcinoma. There are multiple treatment options for CHB, including pegylated-IFNα and reverse-transcriptase inhibitors (Bhattacharya, D., and C. L. Thio. 2010. Review of hepatitis B therapeutics. Clin. Infect. Dis. 51: 1201–1208.), but these treatments are rarely curative (Zhang *et al.* 2016. HBsAg seroclearance or seroconversion induced by peg-interferon alpha and lamivudine or adefovir combination therapy in chronic hepatitis B treatment: a meta-analysis and systematic review. Rev Esp Enferm Dig 108: 263–270.).

[0005] Developing cellular immunotherapeutic strategies for CHB is supported by the fact that 90-95% of acutely HBV-infected adults mount broad, highly functional HBV-specific T cell responses and subsequently clear infection (Maini *et al.* 1999. Direct ex vivo analysis of hepatitis B virus-specific CD8(+) T cells associated with the control of infection. Gastroenterology 117: 1386–1396; Phillips *et al.* 2010. CD8(+) T cell control of hepatitis B virus replication: direct comparison between cytolytic and noncytolytic functions. J. Immunol. 184: 287–295; Fisicaro *et al.* 2009. Early kinetics of innate and adaptive immune responses during hepatitis B virus infection. Gut 58: 974–982.). In contrast, patients progressing to CHB exhibit narrowly-focused, low-frequency, functionally-exhausted HBV-specific T cell responses (Bertoletti, A., and C. Ferrari. 2016. Adaptive immunity in HBV infection. J. Hepatology 64: S71–S83; Rehermann, B., and A. Bertoletti. 2015. Immunological aspects of antiviral therapy of chronic hepatitis B virus and hepatitis C virus infections. Hepatology 61: 712–721; Kurktschiev *et al.* 2014. Dysfunctional CD8+ T cells in hepatitis B and C are characterized by a lack of antigen-specific T-bet induction. J. Exp. Med. 211: 2047–2059.). Therefore, many immunotherapeutic strategies currently in development focus on augmentation of HBV-specific T cell immunity.

[0006] Immunotherapies currently under investigation are designed to harness the immune system to better target HBV infected hepatocytes and include immune stimulation with pattern recognition receptor agonists, check point inhibitor blockades, therapeutic vaccines, and adoptive T cell therapy (Gill, U. S., and P. T. F. Kennedy. 2017. Current therapeutic approaches for HBV infected patients. J. Hepatology 67: 412–414.). A common hurdle facing HBV immunotherapies is T cell immunotolerance (Zong *et al.* 2019. Breakdown of adaptive immunotolerance induces hepatocellular carcinoma in HBsAg-tg mice. Nature Communications 10: 221; Kong *et al.* 2014. γδT cells drive myeloid-derived suppressor cell-mediated CD8+ T cell exhaustion in hepatitis B virus-induced immunotolerance. J. Immunol. 193: 1645–1653; Milich, D. R. 2016. The Concept of Immune Tolerance in Chronic Hepatitis B Virus Infection Is Alive and Well. Gastroenterology 151: 801–804.). The initial triggers of immunotolerance, which distinguishes patients that successfully clear acute HBV viremia from those that do not, is not completely understood. However, it is likely in part a consequence of the immunotolerant environment of the liver. Thus, in order to successfully clear CHB via immunotherapy, T cell immunotolerance must be overcome. Unfortunately, no

immunotherapies to date have consistently achieved this goal, and this reality has been exacerbated by the lack of physiologically relevant animal models of CHB.

[0007] T cell-based immunotherapies for CHB must provide lasting reversal of T cell exhaustion or sustained viral suppression. Given the difficulty in reversing the dysfunction of established HBV-specific T cells in CHB patients, the most effective way to augment HBV-specific T cell immunity may be to engender or impart a completely unique set of T cell responses through therapeutic vaccination or adoptive T cell therapy. Unfortunately, generating such *de novo* responses is limited by patient-specific HLA expression and the HBV peptides that these molecules present on the hepatocyte surface. In contrast, if universally expressed, unconventional MHC-Ib T cell restriction elements that do not contribute to the natural, acute HBV-specific immune response could present HBV antigen on the hepatocyte surface, they could be targeted to elicit a totally distinct set of T cell responses not typically found in HBV infection. Thus, there is an urgent global need to develop curative therapeutics for HBV.

BRIEF SUMMARY OF THE INVENTION

[0008] The present disclosure relates to a method of generating an immune response to a hepatitis B virus (HBV) in a subject, the method comprising administering to the subject a CMV vector expressing a HBV antigen in an amount effective to elicit a CD8+ T cell response to the HBV antigen, wherein the CMV vector does not express an active UL128, UL130, UL146 and UL147 protein or orthologs thereof. In one embodiment, the HBV antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

[0009] The present disclosure also relates to a method of treating chronic HBV infection in a subject, the method comprising administering to the subject a CMV vector expressing a HBV antigen in an amount effective to elicit a CD8+ T cell response to the HBV antigen, wherein the CMV vector does not express an active UL128, UL130, UL146 and UL147 protein or orthologs thereof. In one embodiment, the HBV antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

[0010] The present disclosure also relates to a CMV vector expressing a HBV antigen for use in generating an immune response to a HBV in a subject, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs

thereof. In one embodiment, the HBV antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

[0011] The present disclosure also relates to a CMV vector expressing a HBV antigen for use in the treatment of a chronic HBV infection in a subject, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof. In one embodiment, the HBV antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

[0012] The present disclosure also relates to use of a CMV vector expressing a HBV antigen in the manufacture of a medicament for use in generating an immune response to a HBV in a subject, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof. In one embodiment, the HBV antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

[0013] The present disclosure also relates to use of a CMV vector expressing a HBV antigen in the manufacture of a medicament for the treatment of a chronic HBV infection, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof. In one embodiment, the HBV antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

[0014] In one embodiment, the hepatitis B virus antigens are hepatitis B virus core, envelope, surface, X, or polymerase antigens. In some embodiments, the hepatitis B virus antigen has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identity to PSVRDLLDTASALYR (SEQ ID NO: 17). In some embodiments, the hepatitis B virus antigen has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identity to TALRQAILCWGELMT (SEQ ID NO: 18).

[0015] In another embodiment, at least 10% of the CD8+ T cells elicited by the CMV vector are restricted by MHC-E or an ortholog thereof, or MHC-II or an ortholog thereof. In another embodiment, at least 20%, at least 30%, at least 40%, at least 50%, at least 60% or at least 75% of the CD8+ T cells elicited by the CMV vector are restricted by MHC-E or an ortholog thereof, or MHC-II or an ortholog thereof. In another embodiment, fewer than 10% of the CD8+ T cells elicited by the CMV vector are restricted by MHC-class la or an ortholog thereof. In another embodiment, some of the CD8+ T cells restricted by MHC-E recognize peptides shared by at least 90% of other subjects immunized with the vector. In some embodiments, the CD8+ T cells restricted

by MHC-E recognize a MHC-E supertope. In some embodiments, the MHC-E supertope has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identity to PSVRDLLDTASALYR (SEQ ID NO: 17). In some embodiments, the MHC-E supertope has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identity to TALRQAILCWGELMT (SEQ ID NO: 18).

[0016] The present disclosure also relates to a method of generating CD8+ T cells that recognize MHC-E-HBV antigen peptide complexes, the method comprising: (a) administering to a first subject a recombinant CMV vector comprising a nucleic acid that expresses a HBV antigen, in an amount effective to generate a set of CD8+ T cells that recognize MHC-E/peptide complexes, wherein the CMV vector does not express an active UL128, UL130, UL146 and UL147 protein or orthologs thereof; (b) identifying a first CD8+ TCR from the set of CD8+ T cells, wherein the first CD8+ TCR recognizes a MHC-E/HBV antigen-derived peptide complex; (c) isolating one or more CD8+ T cells from a second subject; and (d) 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 CD8+ T cells that recognize a MHC-E/HBV antigen peptide complex.

[0017] The present disclosure also relates to a method of generating CD8+ T cells that recognize MHC-E-HBV antigen peptide complexes, the method comprising: (a) isolating from a first subject a first set of CD8+ T cells, wherein the first subject has been administered a recombinant CMV vector comprising a nucleic acid that expresses a HBV antigen, in an amount effective to generate a set of CD8+ T cells that recognize MHC-E/peptide complexes, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof; (b) identifying a first CD8+ TCR from the first set of CD8+ T cells, wherein the first CD8+ TCR recognizes a MHC-E/HBV antigen-derived peptide complex; (c) isolating a second set of CD8+ T cells from a second subject; and (d) transfecting the second set of 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 CD8+ T cells that recognize a MHC-E/HBV antigen peptide complex.

[0018] In one embodiment, the recombinant CMV vector is a recombinant human CMV vector or a recombinant rhesus macaque CMV vector. In another embodiment, the hepatitis B virus antigens are hepatitis B virus core, envelope, surface, or polymerase antigens. In some embodiments, the hepatitis B virus antigen has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identity to PSVRDLLDTASALYR (SEQ ID NO: 17). In some embodiments, the hepatitis B virus antigen has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% identity to TALRQAILCWGELMT (SEQ ID NO: 18).

[0019] In one embodiment, the first CD8+ T cell recognizes specific MHC-E supertopes. In another embodiment, the second CD8+ T cell recognizes specific MHC-E supertopes. In some embodiments, the MHC-E supertope is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18). In some embodiments, the MHC-E supertope is PSVRDLLDTASALYR (SEQ ID NO: 17). In some embodiments, the MHC-E supertope is TALRQAILCWGELMT (SEQ ID NO: 18).

[0020] In another embodiment, the first CD8+ TCR is identified by DNA or RNA sequencing. In another embodiment, the nucleic acid sequence encoding the second CD8+ TCR is identical to the nucleic acid sequence encoding the first CD8+ TCR.

[0021] In one embodiment, the first subject is a human or nonhuman primate. In another embodiment, the subject is a nonhuman primate and the second subject is a human, and wherein the second CD8+ TCR is a chimeric nonhuman primate-human CD8+ TCR comprising the non-human primate CDR3α and CDR3β of the first CD8+ TCR. In another embodiment, the second CD8+ TCR comprises the non-human primate CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the first CD8+ TCR. In another embodiment, the second CD8+ TCR comprises CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the first CD8+ TCR. In another embodiment, the nucleic acid sequence encoding the second CD8+ TCR is identical to the nucleic acid sequence encoding the first CD8+ TCR. In another embodiment, the second CD8+ TCR is a chimeric CD8+ TCR. In another embodiment, the second CD8+ TCR comprises CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the first CD8+ TCR.

[0022] In one embodiment, administering the CMV vector to the first subject comprises intravenous, intramuscular, intraperitoneal, or oral administration of the CMV vector to the first subject. In another embodiment, the transfected CD8+ T cells are further administered to the second subject to treat or prevent HBV infection.

[0023] The present disclosure also relates to a CD8+ T cell generated by the methods described herein.

[0024] The present disclosure also relates to a method of treating or preventing a hepatitis B infection in a subject, the method comprising administering a CD8+ T cell described herein to the subject in need thereof. The present disclosure also relates to a CD8+ T cell for use in a method of treating or preventing a hepatitis B infection in a subject in need thereof. The present disclosure also relates to the use of a CD8+ T cell in the manufacture of a medicament in a method of treating or preventing a hepatitis B infection in a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0025] Figure 1A shows the frequency of HBV-antigen specific CD8+ T cell responses of four Rhesus macaques (RM) inoculated with strain 68-1 RhCMV expressing HBV core, surface, and polymerase antigens (RhCMV/HBV 68-1). Figure 1B shows CD8+ T cell response against individual peptides of HBV core antigens (HBcAg) Each HBcAg 15-mer is indicated by a box, color coded as shown to indicate MHC restriction. Figure 1C shows response of CD8+ T cells isolated from inoculated RM to K562 cells transfected with either HLA-E or Mamu-E upon addition of HBcAg peptides.

[0026] Figure 2A shows staining with MHC-E specific antibody 4D12 of MHC-transfected cell lines. 4D12 staining was compared to match IgG-isotype control. In addition, cells were stained with the pan-MHC-I-specific antibody W6/32. Figure 2B shows 4D12 staining of human and RM primary hepatocytes one day after liver perfusion and plating. Mouse IgG1 isotype was used to control for non-specific antibody binding by primary hepatocytes. Figure 2C shows the quantification of the percent of MHC-E+ primary hepatocytes from Fig. 2B. Figure 2D shows co-staining of surface MHC-I, MHC-E, or MHC-II and intracellular HBcAg of human donor primary hepatocytes at four days post-infection with HBV. Figure 2E shows quantification of the percent of HBV+ primary hepatocytes from Fig. 2D.

[0027] Figure 3A shows the percent of HBV-specific CD8+ T cells restricted by MHC-I, MHC-II, and MHC-E in splenocytes and CD8β-sorted effectors from RM1 and RM2 upon incubation with HBV-naïve or HBV-infected PH from two unrelated RM donors (RM8 and RM9). Responding T cells were identified by staining for CD3, CD8, and IFN-γ. MHC restriction of the responding CD8+ T cells was identified with the following MHC blocking agents: W6/32 antibody (pan MHC-I), VL9 peptide (MHC-E), CLIP (MHC-II), or HLA-DR antibody (MHC-II). Figure 3B shows the percent of HBV-specific CD8+ T cells restricted by MHC-I, MHC-II, and MHC-E from splenocytes or CD8β-sorted effectors from RM1 and RM2 that were incubated with HBV-naïve or HBV-infected primary hepatocytes from human donors (HD1 and HD2). Responding CD8+ T cells were identified by CD3, Cd8, and IFN-γ. MHC restriction of the responding CD8+ T cells was also identified with the following MHC blocking agents: W6/32 antibody (pan MHC-I), VL9 peptide (MHC-E), CLIP (MHC-II), or HLA-DR antibody (MHC-II).

[0028] Figure 4 is a bar graph showing the conservation of MHC-E-bound supertopes in HBV core antigen across global HBV strains. 6,203 full genome HBV sequences spanning all known HBV genotypes were translated and amino acids aligned against Core 7 (Fig. 4A) and Core 14 (Fig. 4B).

DETAILED DESCRIPTION OF THE INVENTION

I. Terms

[0029] Unless otherwise noted, technical terms are used according to conventional usage.

[0030] All publications, patents, patent applications, internet sites, and accession numbers/database sequences (including both polynucleotide and polypeptide sequences) cited herein or listed in the Application Data Sheet, including U.S. Provisional Patent Applications No. 62/858,764 filed June 7, 2019, are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, internet site, or accession number/database sequence were specifically and individually indicated to be so incorporated by reference.

[0031] Unless the context requires otherwise, throughout the present specification and claims, the word "comprise" and variations thereof, such as "comprises" and "comprising," are to be construed in an open, inclusive sense, that is, as "including, but not limited to". "Consisting of" shall mean excluding more than trace elements of other

ingredients and substantial method steps disclosed herein. The term "consisting essentially of" limits the scope of a claim to the specified materials or steps, or to those that do not materially affect the basic characteristics of a claimed invention. For example, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. Similarly, a protein consists essentially of a particular amino acid sequence when the protein includes additional amino acids that contribute to at most 20% of the length of the protein and do not substantially affect the activity of the protein (e.g., alters the activity of the protein by no more than 50%). Embodiments defined by each of the transitional terms are within the scope of this invention

[0032] Although methods and materials similar or equivalent to those described herein may be used in the practice or testing of this disclosure, suitable methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided.

[0033] **Antigen:** As used herein, the terms "antigen" or "immunogen" are used interchangeably to refer to a substance, typically a protein, which is capable of inducing an immune response in a subject. The term also refers to proteins that are immunologically active in the sense that once administered to a subject (either directly or by administering to the subject a nucleotide sequence or vector that encodes the protein) the protein is able to evoke an immune response of the humoral and/or cellular type directed against that protein.

[0034] **Antigen-specific T cell:** A CD8+ or CD4+ lymphocyte that recognizes a particular antigen. Generally, antigen-specific T cells specifically bind to a particular antigen presented by MHC molecules, but not other antigens presented by the same MHC.

[0035] **Administration:** As used herein, the term "administration" means to provide or give a subject an agent, such as a composition comprising an effective amount of a CMV vector comprising an exogenous antigen by any effective route. Exemplary routes of administration include, but are not limited to, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, and intravenous), oral, sublingual, rectal, transdermal, intranasal, vaginal, and inhalation routes.

[0036] **Effective amount:** As used herein, the term "effective amount" refers to an amount of an agent, such as a CMV vector comprising a heterologous antigen or a transfected CD8+ T cell that recognizes a MHC-E/heterologous antigen-derived peptide complex, a MHC-II/heterologous antigen-derived peptide complex, or a MHC-I/heterologous antigen-derived peptide complex, that is sufficient to generate a desired response, such as reduce or eliminate a sign or symptom of a condition or disease or induce an immune response to an antigen. In some examples, an "effective amount" is one that treats (including prophylaxis) one or more symptoms and/or underlying causes of any of a disorder or disease. An effective amount may be a therapeutically effective amount, including an amount that prevents one or more signs or symptoms of a particular disease or condition from developing, such as one or more signs or symptoms associated with an infectious disease.

[0037] **Heterologous antigen:** As used herein, the term "heterologous antigen" refers to any protein or fragment thereof that is not derived from CMV. Heterologous antigens may be any antigen derived from HBV.

[0038] **Immunogenic peptide:** A peptide which comprises an allele-specific motif or other sequence, such as an N-terminal repeat, such that the peptide will bind an MHC molecule and induce a cytotoxic T lymphocyte ("CTL") response, or a B cell response (for example antibody production) against the antigen from which the immunogenic peptide is derived.

[0039] In one embodiment, immunogenic peptides are identified using sequence motifs or other methods, such as neural net or polynomial determinations known in the art. Typically, algorithms are used to determine the "binding threshold" of peptides to select those with scores that give them a high probability of binding at a certain affinity and will be immunogenic. The algorithms are based either on the effects on MHC binding of a particular amino acid at a particular position, the effects on antibody binding of a particular amino acid at a particular position, or the effects on binding of a particular substitution in a motif-containing peptide. Within the context of an immunogenic peptide, a "conserved residue" is one which appears in a significantly higher frequency than would be expected by random distribution at a particular position in a peptide. In one embodiment, a conserved residue is one where the MHC structure may provide a contact point with the immunogenic peptide.

[0040] **Mutation:** As used herein, the term "mutation" refers to any difference in a nucleic acid or polypeptide sequence from a normal, consensus, or "wild type" sequence. A mutant is any protein or nucleic acid sequence comprising a mutation. In addition, a cell or an organism with a mutation may also be referred to as a mutant. Some types of coding sequence mutations include point mutations (differences in individual nucleotides or amino acids); silent mutations (differences in nucleotides that do not result in an amino acid changes); deletions (differences in which one or more nucleotides or amino acids are missing, up to and including a deletion of the entire coding sequence of a gene); frameshift mutations (differences in which deletion of a number of nucleotides indivisible by 3 results in an alteration of the amino acid sequence). A mutation that results in a difference in an amino acid may also be called an amino acid substitution mutation. Amino acid substitution mutations may be described by the amino acid change relative to wild type at a particular position in the amino acid sequence.

[0041] **Nucleotide sequences or nucleic acid sequences:** The terms "nucleotide sequences" and "nucleic acid sequences" refer to deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequences, including, without limitation, messenger RNA (mRNA), DNA/RNA hybrids, or synthetic nucleic acids. The nucleic acid may be single-stranded, or partially or completely double stranded (duplex). Duplex nucleic acids may be homoduplex or heteroduplex.

[0042] **Operably Linked:** As the term "operably linked" is used herein, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in such a way that it has an effect upon the second nucleic acid sequence. Operably linked DNA sequences may be contiguous, or they may operate at a distance.

[0043] **Promoter:** As used herein, the term "promoter" may refer to any of a number of nucleic acid control sequences that directs transcription of a nucleic acid. Typically, a eukaryotic promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element or any other specific DNA sequence that is recognized by one or more transcription factors. Expression by a promoter may be further modulated by enhancer or repressor elements. Numerous examples of promoters are available and well known to those of ordinary skill in the art. A nucleic acid comprising a promoter operably linked to a nucleic acid sequence that codes for a particular polypeptide may be termed an expression vector.

[0044] **Recombinant:** As used herein, the term "recombinant" with reference to a nucleic acid or polypeptide refers to one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence, for example a CMV vector comprising a heterologous antigen. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, *e.g.*, by genetic engineering techniques. A recombinant polypeptide may also refer to a polypeptide that has been made using recombinant nucleic acids, including recombinant nucleic acids transferred to a host organism that is not the natural source of the polypeptide (for example, nucleic acids encoding polypeptides that form a CMV vector comprising a heterologous antigen).

[0045] **Pharmaceutically acceptable carriers:** As used herein, a "pharmaceutically acceptable carrier" of use is conventional. Remington's Pharmaceutical Sciences, by E.W. Martin, Mack Publishing Co., Easton, PA, 19th Edition, 1995, describes compositions and formulations suitable for pharmaceutical delivery of the compositions disclosed herein. In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol, or the like as a vehicle. For solid compositions (such as powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers may include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered may contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

[0046] **Polynucleotide:** As used herein, the term "polynucleotide" refers to a polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). A polynucleotide is made up of four bases; adenine, cytosine, guanine, and thymine/uracil (uracil is used in RNA). A coding sequence from a nucleic acid is indicative of the sequence of the protein encoded by the nucleic acid.

[0047] **Polypeptide:** The terms "protein", "peptide", "polypeptide", and "amino acid sequence" are used interchangeably herein to refer to polymers of amino acid residues of any length. The polymer may be linear or branched, it may comprise modified amino

acids or amino acid analogs, and it may be interrupted by chemical moieties other than amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling or bioactive component.

[0048] Orthologs of proteins are typically characterized by possession of greater than 75% sequence identity counted over the full-length alignment with the amino acid sequence of specific protein using ALIGN set to default parameters. Proteins with even greater similarity to a reference sequence will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, or at least 98% sequence identity. In addition, sequence identity can be compared over the full length of particular domains of the disclosed peptides.

[0049] **Sequence identity/similarity:** As used herein, the identity/similarity between two or more nucleic acid sequences, or two or more amino acid sequences, is expressed in terms of the identity or similarity between the sequences. Sequence identity may be measured in terms of percentage identity; the higher the percentage, the more identical the sequences are. Sequence similarity may be measured in terms of percentage identity or similarity (which takes into account conservative amino acid substitutions); the higher the percentage, the more similar the sequences are. Polypeptides or protein domains thereof that have a significant amount of sequence identity and also function the same or similarly to one another (for example, proteins that serve the same functions in different species or mutant forms of a protein that do not change the function of the protein or the magnitude thereof) may be called "homologs."

[0050] Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith & Waterman, Adv Appl Math 2, 482 (1981); Needleman & Wunsch, J Mol Biol 48, 443 (1970); Pearson & Lipman, Proc Natl Acad Sci USA 85, 2444 (1988); Higgins & Sharp, Gene 73, 237-244 (1988); Higgins & Sharp, CABIOS 5, 151-153 (1989); Corpet *et al.*, Nuc Acids Res 16, 10881-10890 (1988); Huang *et al.*, Computer App Biosci 8, 155-165 (1992); and Pearson *et al.*, Meth Mol Bio 24,307-331 (1994). In addition, Altschul *et al.*, J Mol Biol 215, 403-410 (1990), presents a detailed consideration of sequence alignment methods and homology calculations.

[0051] The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, (1990) supra) is available from several sources, including the National Center for Biological Information (NCBI, National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. Additional information may be found at the NCBI web site.

[0052] BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences.

[0053] Once aligned, the number of matches is determined by counting the number of positions where an identical nucleotide or amino acid residue is presented in both sequences. The percent sequence identity is determined by dividing the number of matches either by the length of the sequence set forth in the identified sequence, or by an articulated length (such as 100 consecutive nucleotides or amino acid residues from a sequence set forth in an identified sequence), followed by multiplying the resulting value by 100. For example, a nucleic acid sequence that has 1166 matches when aligned with a test sequence having 1154 nucleotides is 75.0 percent identical to the test sequence (1166÷1554*100=75.0). The percent sequence identity value is rounded to the nearest tenth. For example, 75.11, 75.12, 75.13, and 75.14 are rounded down to 75.1, while 75.15, 75.16, 75.17, 75.18, and 75.19 are rounded up to 75.2. The length value will always be an integer. In another example, a target sequence containing a 20-nucleotide region that aligns with 20 consecutive nucleotides from an identified sequence as follows contains a region that shares 75 percent sequence identity to that identified sequence (that is, 15÷20*100=75).

[0054] For comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). Homologs are typically characterized by possession of at least 70% sequence identity counted over the full-length alignment with an amino acid sequence using the NCBI Basic Blast 2.0, gapped blastp with databases such as the nr database, swissprot database, and patented sequences database. Queries searched with the blastn program are filtered with DUST

(Hancock & Armstrong, Comput Appl Biosci 10, 67-70 (1994.) Other programs use SEG. In addition, a manual alignment may be performed. Proteins with even greater similarity will show increasing percentage identities when assessed by this method, such as at least about 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to a protein.

[0055] When aligning short peptides (fewer than around 30 amino acids), the alignment is performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequence will show increasing percentage identities when assessed by this method, such as at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to a protein. When less than the entire sequence is being compared for sequence identity, homologs will typically possess at least 75% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85%, 90%, 95% or 98% depending on their identity to the reference sequence. Methods for determining sequence identity over such short windows are described at the NCBI web site.

[0056] One indication that two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions, as described above. Nucleic acid sequences that do not show a high degree of identity may nevertheless encode identical or similar (conserved) amino acid sequences, due to the degeneracy of the genetic code. Changes in a nucleic acid sequence may be made using this degeneracy to produce multiple nucleic acid molecules that all encode substantially the same protein. Such homologous nucleic acid sequences can, for example, possess at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% sequence identity to a nucleic acid that encodes a protein.

[0057] **Subject:** As used herein, the term "subject" refers to a living multi-cellular vertebrate organism, a category that includes both human and non-human mammals.

[0058] **Supertope:** As used herein, the term "supertope" or "supertope peptide" refers to a epitope or peptide that is recognized by T cells in greater than about 90% of the human population regardless of MHC haplotype, *i.e.*, in the presence or absence of given MHC-I, MHC-II, or MHC-E alleles.

[0059] **Treatment:** As used herein, the term "treatment" refers to an intervention that ameliorates a sign or symptom of a disease or pathological condition. As used herein, the terms "treatment", "treat", and "treating," with reference to a disease, pathological

condition or symptom, also refers to any observable beneficial effect of the treatment. The beneficial effect may be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, a reduction in the number of relapses of the disease, an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular disease. A prophylactic treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs, for the purpose of decreasing the risk of developing pathology. A therapeutic treatment is a treatment administered to a subject after signs and symptoms of the disease have developed.

[0060] **Vaccine:** An immunogenic composition that can be administered to a mammal, such as a human, to confer immunity, such as active immunity, to a disease or other pathological condition. Vaccines can be used prophylactically or therapeutically. Thus, vaccines can be used reduce the likelihood of developing a disease (such as a tumor or pathological infection) or to reduce the severity of symptoms of a disease or condition, limit the progression of the disease or condition (such as a tumor or a pathological infection), or limit the recurrence of a disease or condition (such as a tumor). In particular embodiments, a vaccine is a replication-deficient CMV expressing a HBV antigen.

[0061] **Vector:** Nucleic acid molecules of particular sequence can be incorporated into a vector that is then introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art, including promoter elements that direct nucleic acid expression. Vectors can be viral vectors, such as CMV vectors. Viral vectors may be constructed from wild type or attenuated virus, including replication deficient virus.

II. Methods for the Treatment and Prevention of Hepatitis B Infection

[0062] Disclosed herein are methods for the treatment or prevention of hepatitis B virus infection. The methods involve administering an effective amount of at least one recombinant CMV vector comprising at least one heterologous antigen to a subject, wherein the at least one heterologous antigen comprises an antigen derived from the hepatitis B virus.

[0063] The antigen derived from the hepatitis B virus may be derived from any portion of the viral pathogen. Hepatitis B antigens include, but are not limited to, the core protein, envelope protein, surface proteins, X protein, and polymerase protein.

[0064] In some embodiments, the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein due to the presence of a mutation in the nucleic acid sequence encoding UL128, UL130, UL146, and UL147 or homologs thereof, or orthologs thereof (homologous genes of CMV that infect other species). The mutation may be any mutation that results in a lack of expression of active proteins. Such mutations may include point mutations, frameshift mutations, deletions of less than all of the sequence that encodes the protein (truncation mutations), or deletions of all of the nucleic acid sequence that encodes the protein, or any other mutations.

[0065] In further examples, the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein due to the presence of a nucleic acid sequence in the vector that comprises an antisense or RNAi sequence (siRNA or miRNA) that inhibits the expression of the UL128, UL130, UL146, and UL147 proteins. Mutations and/or antisense and/or RNAi may be used in any combination to generate a CMV vector lacking active UL128, UL130, UL146, and UL147.

[0066] In some embodiments, the CD8+ T cell response elicited by this vector is characterized by having at least 10% of the CD8+ T cells directed against HBV epitopes presented by MHC-E. In further examples, at least 15%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 75%, at least 90%, or at least 95% of the CD8+ T cells are restricted by MHC-E. In some embodiments, the HBV-specific CD8+ T cells restricted by MHC-E recognize peptides shared by at least 90% of other subjects immunized with the vector. In some embodiments, the CD8+ T cells are directed against a HBV supertope presented by MHC-E. In some embodiments, the CD8+ T cell response elicited by this vector is characterized by having at least 10% of the CD8+ T cells directed against epitopes presented by MHC-II. In further examples, at least 15%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 75%, at least 90%, or at least 95% of the CD8+ T cells are restricted by MHC-II. In some embodiments, the HBV-specific CD8+ T cells restricted by MHC-II recognize HBV peptides shared by at least 90% of other subjects immunized with the vector. In some embodiments, the HBV-specific CD8+ T cells are directed against a HBV supertope presented by MHC-II.

[0067] In some embodiments, the method further comprises identifying a CD8+ T cell receptor from the CD8+ T cells elicited by the CMV vector, wherein the CD8+ T cell receptor recognizes a MHC-E/HBV antigen-derived peptide complex. In some embodiments, the CD8+ T cell receptor is identified by RNA or DNA sequencing. In some embodiments, the method further comprises a CD8+ T cell receptor that recognizes MHC-E supertopes of HBV.

[0068] In some embodiments, the method further comprises identifying a CD8+ T cell receptor from the CD8+ T cells elicited by the CMV vector, wherein the CD8+ T cell receptor recognizes a MHC-II/HBV antigen-derived peptide complex. In some embodiments, the CD8+ T cell receptor is identified by RNA or DNA sequencing. In some embodiments, the method further comprises a CD8+ T cell receptor that recognizes MHC-II supertopes of HBV.

[0069] Also disclosed herein is a method of generating CD8+ T cells that recognize MHC-E-HBV peptide complexes. This method involves administering to a first subject (or animal) a CMV vector in an amount effective to generate a set of CD8+ T cells that recognize MHC-E/HBV peptide complexes. In some embodiments, the CMV vector comprises a first nucleic acid sequence encoding at least one HBV antigen and does not express an active UL128, UL130, UL146, and UL147 proteins or orthologs thereof. In some embodiments, the HBV antigens can be hepatitis B virus core, envelope, surface, or polymerase antigens.

[0070] This method further comprises: identifying a first CD8+ T cell receptor from the set of CD8+ T cells, wherein the first CD8+ T cell receptor recognizes an MHC-E/HBV antigen-derived peptide complex. In some embodiments, the first CD8+ T cell receptor is identified by DNA or RNA sequencing. In some embodiments, this method can further comprise 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+ T cell receptor and a promoter operably linked to the nucleic acid sequence encoding the 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/HBV antigen-derived peptide complex. The one or more CD8+ T cells for transfection with the expression vector may be isolated from the first subject or a second subject.

[0071] In some embodiments, the method further comprises identifying a CD8+ T cell receptor from the CD8+ T cells elicited by the CMV vector, wherein the CD8+ T cell receptor recognizes an MHC-E/HBV antigen-derived peptide complex. In some embodiments, the CD8+ T cell receptor is identified by RNA or DNA sequencing. In some embodiments, the method further comprises an HBV-specific CD8+ T cell receptor that recognizes MHC-E supertopes.

[0072] Also disclosed is a transfected CD8+ T cell that recognizes MHC-E-HBV 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/HBV peptide complexes, wherein the recombinant CMV vector comprises at least one HBV antigen; (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/HBV antigen-derived peptide 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-HBV peptide complexes. The CMV vector comprises a first nucleic acid sequence encoding at least one HBV antigen and does not express an active UL128, UL130, UL146, and UL147 protein or ortholog thereof. The expression vector comprises a nucleic acid sequence encoding a second CD8+ T cell receptor and a promoter operably linked to the nucleic acid sequence encoding the second CD8+ T cell receptor, wherein the second CD8+ T cell receptor comprises CDR3α and CDR3β of the first CD8+ T cell receptor. The hepatitis B antigens may be hepatitis B virus core, envelope, surface, or polymerase antigens.

[0073] In some embodiments, the CD8+ T cell response elicited by the CMV vector is characterized by having at least 10% of the CD8+ T cells directed against HBV epitopes presented by MHC-II. In further examples, at least 15%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 75%, at least 90%, at least 95% or at least 95% of the CD8+ T cells are restricted by MHC-II. In some embodiments, the CD8+ T cells restricted by MHC-II recognize HBV peptides shared by at least 90% of other subjects immunized with the vector. In some embodiments, the CD8+ T cells are directed against a HBV supertope presented by MHC-II.

[0074] In some embodiments, the method further comprises identifying a CD8+ T cell receptor from the CD8+ T cells elicited by the CMV/HBV vector, wherein the CD8+ T

cell receptor recognizes a MHC-II/HBV antigen-derived peptide complex. In some embodiments, the CD8+ T cell receptor is identified by RNA or DNA sequencing. In some embodiments, the method further comprises a CD8+ T cell receptor that recognizes MHC-II-restricted HBV supertopes.

[0075] Human or animal CMV vectors, when used as expression vectors, are innately non-pathogenic in the selected subjects such as humans. In some embodiments, the CMV vectors have been modified to render them non-pathogenic (incapable of within host or host-to-host spread) in the selected subjects.

[0076] A HBV antigen, as described herein, may be any HBV protein or fragment thereof.

[0077] The recombinant CMV vectors disclosed herein may be derived from human cytomegalovirus vectors, rhesus macaque cytomegalovirus vectors, or cynomolgus macaque vectors.

[0078] The recombinant CMV vectors disclosed herein may be used as an immunogenic, immunological or vaccine composition containing the recombinant CMV virus or vector, and a pharmaceutically acceptable carrier or diluent. An immunological composition containing the recombinant CMV virus or vector (or an expression product thereof) elicits an immunological response--local or systemic. The response can, but need not be, protective. An immunogenic composition containing the recombinant CMV virus or vector (or an expression product thereof) likewise elicits a local or systemic immunological response which can, but need not be, protective. A vaccine composition elicits a local or systemic protective response. Accordingly, the terms "immunological composition" and "immunogenic composition" include a "vaccine composition" (as the two former terms may be protective compositions).

[0079] The recombinant CMV vectors disclosed herein may be used in methods of inducing an immunological response in a subject comprising administering to the subject an immunogenic, immunological or vaccine composition comprising the recombinant CMV virus or vector and a pharmaceutically acceptable carrier or diluent.

[0080] The CMV vectors disclosed herein may be used in therapeutic compositions containing the recombinant CMV virus or vector and a pharmaceutically acceptable carrier or diluent. The CMV vectors disclosed herein may be prepared by inserting DNA comprising a sequence that encodes the HBV antigen into an essential or non-essential region of the CMV genome. The method may further comprise deleting one or more

regions from the CMV genome. The method may comprise *in vivo* recombination. Thus, the method may comprise transfecting a cell with CMV DNA in a cell-compatible medium in the presence of donor DNA comprising the heterologous DNA flanked by DNA sequences homologous with portions of the CMV genome, whereby the heterologous DNA is introduced into the genome of the CMV, and optionally then recovering CMV modified by the *in vivo* recombination. The method may also comprise cleaving CMV DNA to obtain cleaved CMV DNA, ligating the heterologous DNA to the cleaved CMV DNA to obtain hybrid CMV-heterologous DNA, transfecting a cell with the hybrid CMV -heterologous DNA, and optionally then recovering CMV modified by the presence of the HBV DNA. Since *in vivo* recombination is comprehended, the method accordingly also provides a plasmid comprising donor DNA not naturally occurring in CMV encoding a polypeptide foreign to CMV, the donor DNA is within a segment of CMV DNA that would otherwise be co-linear with an essential or non-essential region of the CMV genome such that DNA from an essential or nonessential region of CMV is flanking the donor DNA The HBV DNA may be inserted into CMV to generate the recombinant CMV in any orientation that yields stable integration of that DNA, and expression thereof, when desired.

[0081] The DNA encoding the HBV antigen in the recombinant CMV vector may also include a promoter. The promoter may be from any source such as a herpes virus, including an endogenous cytomegalovirus (CMV) promoter, such as a human CMV (HCMV), rhesus macaque CMV (RhCMV), murine, or other CMV promoter. The promoter may also be a nonviral promoter such as the EF1α promoter. The promoter may be a truncated transcriptionally active promoter which comprises a region transactivated with a transactivating protein provided by the virus and the minimal promoter region of the full-length promoter from which the truncated transcriptionally active promoter is derived. The promoter may be composed of an association of DNA sequences corresponding to the minimal promoter and upstream regulatory sequences. A minimal promoter is composed of the CAP site plus ATA box (minimum sequences for basic level of transcription; unregulated level of transcription); "upstream regulatory sequences" are composed of the upstream element(s) and enhancer sequence(s). Further, the term "truncated" indicates that the full-length promoter is not completely present, *i.e.*, that some portion of the full-length promoter has been removed. And, the truncated promoter may be derived from a herpesvirus such as MCMV or HCMV, *e.g.*, HCMV-IE or

MCMV-IE. There may be up to a 40% and even up to a 90% reduction in size, from a full-length promoter, based upon base pairs. The promoter may also be a modified non-viral promoter. As to HCMV promoters, reference is made to U.S. Pat. Nos. 5,168,062 and 5,385,839. As to transfecting cells with plasmid DNA for expression therefrom, reference is made to Feigner *et al.* (1994), *J Biol. Chem.* 269, 2550-2561. And, as to direct injection of plasmid DNA as a simple and effective method of vaccination against a variety of infectious diseases reference is made to *Science,* 259:1745-49, 1993. It is therefore within the scope of this disclosure that the vector may be used by the direct injection of vector DNA.

[0082] Also disclosed is an expression cassette that may be inserted into a recombinant virus or plasmid comprising the truncated transcriptionally active promoter. The expression cassette may further include a functional truncated polyadenylation signal; for instance an SV40 polyadenylation signal which is truncated, yet functional. Considering that nature provided a larger signal, it is indeed surprising that a truncated polyadenylation signal is functional. A truncated polyadenylation signal addresses the insert size limit problems of recombinant viruses such as CMV. The expression cassette may also include HBV DNA with respect to the virus or system into which it is inserted; and that DNA may be HBV DNA as described herein.

[0083] As to HBV antigens for use in vaccine or immunological compositions, see also *Stedman's Medical Dictionary* (24th edition, 1982, *e.g.,* definition of vaccine (for a list of antigens used in vaccine formulations); such antigens or epitopes of interest from those antigens may be used. As to HBV antigens, one skilled in the art may select an antigen and the coding DNA therefor from the knowledge of the amino acid and corresponding DNA sequences of the peptide or polypeptide, as well as from the nature of particular amino acids *(e.g.,* size, charge, etc.) and the codon dictionary, without undue experimentation. Exemplary antigens include, but are not limited to, a hepatitis B virus core, envelope, surface, X, or polymerase antigen.

[0084] One method to determine T epitopes of a HBV antigen involves epitope mapping. Overlapping peptides of the heterologous antigen are generated by oligo-peptide synthesis. The individual peptides are then tested for their ability to bind to an antibody elicited by the native protein or to induce T cell or B cell activation. This approach has been particularly useful in mapping T cell epitopes since the T cell recognizes short linear peptides complexed with MHC molecules.

[0085] An immune response to a HBV antigen is generated, in general, as follows: T cells recognize proteins only when the protein has been cleaved into smaller peptides and is presented in a complex called the "major histocompatibility complex (MHC)" located on another cell's surface. There are two classes of MHC complexes--class I and class II, and each class is made up of many different alleles. Different species, and individual subjects have different types of MHC complex alleles; they are said to have a different MHC type. One type of MHC class I molecule is called MHC-E (HLA-E in humans, Mamu-E in RM, Qa-lb in mice).

[0086] It is noted that the DNA comprising the sequence encoding the HBV antigen may itself include a promoter for driving expression in the CMV vector or the DNA may be limited to the coding DNA of the heterologous antigen. This construct may be placed in such an orientation relative to an endogenous CMV promoter that it is operably linked to the promoter and is thereby expressed. Further, multiple copies of DNA encoding the heterologous antigen or use of a strong or early promoter or early and late promoter, or any combination thereof, may be done so as to amplify or increase expression. Thus, the DNA encoding the heterologous antigen may be suitably positioned with respect to a CMV endogenous promoter, or those promoters may be translocated to be inserted at another location together with the DNA encoding the heterologous antigen. Nucleic acids encoding more than one heterologous antigen may be packaged in the CMV vector.

[0087] Further disclosed are pharmaceutical and other compositions containing the disclosed CMV vectors. Such pharmaceutical and other compositions may be formulated so as to be used in any administration procedure known in the art. Such pharmaceutical compositions may be *via* a parenteral route (intradermal, intramuscular, subcutaneous, intravenous, or others). The administration may also be *via* a mucosal route, *e.g.,* oral, nasal, genital, etc.

[0088] The disclosed pharmaceutical compositions may be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical arts. Such compositions may be administered in dosages and by techniques well known to those skilled in the medical arts taking into consideration such factors as the breed or species, age, sex, weight, and condition of the particular patient, and the route of administration. The compositions may be administered alone, or may be co-administered or sequentially administered with other CMV vectors or with other immunological, antigenic or vaccine or therapeutic compositions. Such other compositions may include purified native

antigens or epitopes or antigens or epitopes from the expression by a recombinant CMV or another vector system; and are administered taking into account the aforementioned factors.

[0089] Examples of compositions include liquid preparations for orifice, *e.g.,* oral, nasal, anal, genital, *e.g.,* vaginal, etc., administration such as suspensions, syrups, or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular, or intravenous administration *(e.g.,* injectable administration) such as sterile suspensions or emulsions. In such compositions the recombinant may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like.

[0090] Antigenic, immunological or vaccine compositions typically may contain an adjuvant and an amount of the CMV vector or expression product to elicit the desired response. In human applications, alum (aluminum phosphate or aluminum hydroxide) is a typical adjuvant. Saponin and its purified component Quil A, Freund's complete adjuvant and other adjuvants used in research and veterinary applications have toxicities which limit their potential use in human vaccines. Chemically defined preparations such as muramyl dipeptide, monophosphoryllipid A, phospholipid conjugates such as those described by Goodman-Snitkoff *et al., J Immunol.* 147:410-415 (1991), encapsulation of the protein within a proteoliposome as described by Miller *et al., J Exp. Med.* 176:1739-1744 (1992), and encapsulation of the protein in lipid vesicles such as Novasome lipid vesicles (Micro Vescular Systems, Inc., Nashua, N.H.) may also be used.

[0091] The composition may be packaged in a single dosage form for immunization by parenteral *(e.g.,* intramuscular, intradermal or subcutaneous) administration or orifice administration, *e.g.,* perlingual *(e.g.,* oral), intragastric, mucosal including intraoral, intraanal, intravaginal, and the like administration. And again, the effective dosage and route of administration are determined by the nature of the composition, by the nature of the expression product, by expression level if recombinant CMV is directly used, and by known factors, such as breed or species, age, sex, weight, condition and nature of host, as well as LD_{50} and other screening procedures which are known and do not require undue experimentation. Dosages of expressed product may range from a few to a few hundred micrograms, *e.g.,* 5 to 500 μg. The CMV vector may be administered in any suitable amount to achieve expression at these dosage levels. In nonlimiting examples: CMV vectors may be administered in an amount of at least 10^2 pfu; thus, CMV vectors may be administered in at least this amount; or in a range from about 10^2 pfu to about 10^7 pfu.

Other suitable carriers or diluents may be water or a buffered saline, with or without a preservative. The CMV vector may be lyophilized for resuspension at the time of administration or may be in solution. "About" may mean within 1%, 5%, 10% or 20% of a defined value.

[0092] It should be understood that the proteins and the nucleic acids encoding them of the present disclosure may differ from the exact sequences illustrated and described herein. Thus, the disclosure contemplates deletions, additions, truncations, and substitutions to the sequences shown, so long as the sequences function in accordance with the methods of the disclosure. In this regard, substitutions will generally be conservative in nature, *i.e.*, those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic--aspartate and glutamate; (2) basic--lysine, arginine, and histidine; (3) nonpolar-- alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan; and (4) uncharged polar--glycine, asparagine, glutamine, cysteine, serine threonine, and tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. It is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, or vice versa; an aspartate with a glutamate or vice versa; a threonine with a serine or vice versa; or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the proteins described but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of the protein are, therefore, within the scope of the disclosure.

[0093] The nucleotide sequences of the present disclosure may be codon optimized, for example the codons may be optimized for use in human cells. For example, any viral or bacterial sequence may be so altered. Many viruses, including HIV and other lentiviruses, use a large number of rare codons and, by altering these codons to correspond to codons commonly used in the desired subject, enhanced expression of the heterologous antigen may be achieved as described in Andreetal., *J Virol.* 72:1497-1503,1998.

[0094] Nucleotide sequences encoding functionally and/or antigenically equivalent variants and derivatives of the CMV vectors and the glycoproteins included therein are contemplated. These functionally equivalent variants, derivatives, and fragments display the ability to retain antigenic activity. For instance, changes in a DNA sequence that do not change the encoded amino acid sequence, as well as those that result in conservative

substitutions of amino acid residues, one or a few amino acid deletions or additions, and substitution of amino acid residues by amino acid analogs are those which will not significantly affect properties of the encoded polypeptide. Conservative amino acid substitutions are glycine/alanine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; serine/threonine/methionine; lysine/arginine; and phenylalanine/tyrosine/tryptophan. In one embodiment, the variants have at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homology or identity to the antigen, epitope, immunogen, peptide, or polypeptide of interest.

[0095] Sequence identity or homology is determined by comparing the sequences when aligned so as to maximize overlap and identity while minimizing sequence gaps. In particular, sequence identity may be determined using any of a number of mathematical algorithms. A nonlimiting example of a mathematical algorithm used for comparison of two sequences is the algorithm of Karlin & Altschul, *Proc. Natl. Acad. Sci. USA* 1990; 87: 2264-2268, modified as in Karlin & Altschul, *Proc. Natl. Acad. Sci. USA* 1993;90: 5873-5877.

[0096] Another example of a mathematical algorithm used for comparison of sequences is the algorithm of Myers & Miller, *CABIOS* 1988;4: 11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 may be used. Yet another useful algorithm for identifying regions of local sequence similarity and alignment is the FASTA algorithm as described in Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* 1988; 85: 2444-2448.

[0097] Advantageous for use according to the present disclosure is the WU –BLAST (Washington University BLAST) version 2.0 software. WU-BLAST version 2.0 executable programs for several UNIX platforms may be downloaded from ftp://blast.wustl.edu/blast/executables. This program is based on WU-BLAST version 1.4, which in tum is based on the public domain NCBI -BLAST version 1.4 (Altschul & Gish, 1996, Local alignment statistics, Doolittle ed., *Methods in Enzymology* 266: 460- 480; Altschul *et al., Journal of Molecular Biology* 1990; 215: 403-410; Gish & States, 1993;

Nature Genetics 3: 266-272; Karlin & Altschul, 1993; *Proc. Natl. Acad. Sci. USA* 90: 5873-5877; all of which are incorporated by reference herein).

[0098] The various recombinant nucleotide sequences and antibodies and/or antigens of the disclosure are made using standard recombinant DNA and cloning techniques. Such techniques are well known to those of skill in the art. See for example, *"Molecular Cloning: A Laboratory Manual"*, second edition (Sambrook *et al.* 1989).

[0099] The nucleotide sequences of the present disclosure may be inserted into "vectors." The term "vector" is widely used and understood by those of skill in the art, and as used herein the term "vector" is used consistent with its meaning to those of skill in the art. For example, the term "vector" is commonly used by those skilled in the art to refer to a vehicle that allows or facilitates the transfer of nucleic acid molecules from one environment to another or that allows or facilitates the manipulation of a nucleic acid molecule.

[0100] Any vector that allows expression of the viruses of the present disclosure may be used in accordance with the present disclosure. In certain embodiments, the disclosed viruses may be used *in vitro* (such as using cell-free expression systems) and/or in cultured cells grown *in vitro* in order to produce the encoded heterologous antigen *(e.g.,* pathogen-specific antigens, HIV antigens, hepatitis B antigens, and antibodies) which may then be used for various applications such as in the production of proteinaceous vaccines. For such applications, any vector that allows expression of the virus *in vitro* and/or in cultured cells may be used.

[0101] For the disclosed heterologous antigens to be expressed, the protein coding sequence of the heterologous antigen should be "operably linked" to regulatory or nucleic acid control sequences that direct transcription and translation of the protein. As used herein, a coding sequence and a nucleic acid control sequence or promoter are said to be "operably linked" when they are covalently linked in such a way as to place the expression or transcription and/or translation of the coding sequence under the influence or control of the nucleic acid control sequence. The "nucleic acid control sequence" may be any nucleic acid element, such as, but not limited to promoters, enhancers, IRES, introns, and other elements described herein that direct the expression of a nucleic acid sequence or coding sequence that is operably linked thereto. The term "promoter" will be used herein to refer to a group of transcriptional control modules that are clustered around the initiation site for RNA polymerase II and that when operationally linked to the protein

coding sequences of the disclosure lead to the expression of the encoded protein. The expression of the transgenes of the present disclosure may be under the control of a constitutive promoter or of an inducible promoter, which initiates transcription only when exposed to some particular external stimulus, such as, without limitation, antibiotics such as tetracycline, hormones such as ecdysone, or heavy metals. The promoter may also be specific to a particular cell-type, tissue, or organ. Many suitable promoters and enhancers are known in the art, and any such suitable promoter or enhancer may be used for expression of the transgenes of the disclosure. For example, suitable promoters and/or enhancers may be selected from the Eukaryotic Promoter Database (EPDB).

[0102] The vectors used in accordance with the present disclosure may contain a suitable gene regulatory region, such as a promoter or enhancer, such that the antigens of the disclosure may be expressed.

[0103] The CMV vectors described herein may contain mutations that may prevent host to host spread, thereby rendering the virus unable to infect immunocompromised or other subjects that could face complications as a result of CMV infection. The CMV vectors described herein may also contain mutations that result in the presentation of immunodominant and nonimmunodominant epitopes as well as non-canonical MHC restriction. However, mutations in the CMV vectors described herein do not affect the ability of the vector to reinfect a subject that has been previously infected with CMV. Such CMV mutations are described in, for example, US Patent Publications 2013-013676S; 2010-0142S23; 2014-014103S; and PCT application publication WO 2014/13S209, all of which are incorporated by reference herein.

[0104] The disclosed CMV vectors may be administered *in vivo,* for example where the aim is to produce an immunogenic response, including a CD8+ immune response, including an immune response characterized by a high percentage of the CD8+ T cell response being restricted by MHC-E or MHC-II(or a homolog or ortholog thereof). For example, in some examples it may be desired to use the disclosed CMV vectors in a laboratory animal, such as rhesus macaques for preclinical testing of immunogenic compositions and vaccines using RhCMV. In other examples, it will be desirable to use the disclosed CMV vectors in human subjects, such as in clinical trials and for actual clinical use of the immunogenic compositions using HCMV.

[0105] For such *in vivo* applications the disclosed CMV vectors are administered as a component of an immunogenic composition further comprising a pharmaceutically

acceptable carrier. In some embodiments, the immunogenic compositions of the disclosure are useful to stimulate an immune response against the heterologous antigen, including a pathogen-specific antigen and may be used as one or more components of a prophylactic or therapeutic vaccine against pathogen-specific antigens for the prevention, amelioration, or treatment of a pathogenic infection. The nucleic acids and vectors of the disclosure are particularly useful for providing genetic vaccines, *i.e.,* vaccines for delivering the nucleic acids encoding the antigens of the disclosure to a subject, such as a human, such that the antigens are then expressed in the subject to elicit an immune response.

[0106] Immunization schedules (or regimens) are well known for animals (including humans) and may be readily determined for the particular subject and immunogenic composition. Hence, the immunogens may be administered one or more times to the subject. Preferably, there is a set time interval between separate administrations of the immunogenic composition. While this interval varies for every subject, typically it ranges from 10 days to several weeks, [and is often 2, 4, 6, or 8 weeks. For humans, the interval is typically from 2 to 6 weeks. In a particularly advantageous embodiment of the present disclosure, the interval is longer, advantageously about 10 weeks, 12 weeks, 14 weeks, 16 weeks, 18 weeks, 20 weeks, 22 weeks, 24 weeks, 26 weeks, 28 weeks, 30 weeks, 32 weeks, 34 weeks, 36 weeks, 38 weeks, 40 weeks, 42 weeks, 44 weeks, 46 weeks, 48 weeks, 50 weeks, 52 weeks, 54 weeks, 56 weeks, 58 weeks, 60 weeks, 62 weeks, 64 weeks, 66 weeks, 68 weeks, or 70 weeks. The immunization regimes typically have from 1 to 6 administrations of the immunogenic composition, but may have as few as one or two or four. The methods of inducing an immune response may also include administration of an adjuvant with the immunogens. In some instances, annual, biannual or other long interval (5-10 years) booster immunization may supplement the initial immunization protocol. The present methods also include a variety of prime-boost regimens. In these methods, one or more priming immunizations are followed by one or more boosting immunizations. The actual immunogenic composition may be the same or different for each immunization and the type of immunogenic composition (*e.g.*, containing protein or expression vector), the route, and formulation of the immunogens may also be varied. For example, if an expression vector is used for the priming and boosting steps, it may either be of the same or different type (*e.g.*, DNA or bacterial or viral expression vector). One useful prime-boost regimen provides for two priming

immunizations, four weeks apart, followed by two boosting immunizations at 4 and 8 weeks after the last priming immunization. It should also be readily apparent to one of skill in the art that there are several permutations and combinations that are encompassed using the DNA, bacterial and viral expression vectors of the disclosure to provide priming and boosting regimens. CMV vectors may be used repeatedly while expressing different antigens derived from different pathogens.

EXAMPLES

EXAMPLE 1: RHCMV/HBV INOCULATED RHESUS MACAQUES MOUNT MHC-E RESTRICTED CD8+ T CELL RESPONSES AGAINST HBV ANTIGENS

[0107] RhCMV strain 68-1 vectors engineered to express antigenic targets elicit broad, effector-memory CD8+ T cell responses restricted either by the non-classical molecule MHC-E, a monomorphic MHC class Ib molecule normally involved in NK cell signaling, or MHC-II. RhCMV vaccine vectors expressing simian immunodeficiency virus (SIV) antigens protect 50% of RM following repeated low-dose intrarectal and intravaginal challenge with the highly pathogenic strain SIVmac239.

[0108] Here, it was determined whether RhCMV/HBV-induced, unconventionally restricted CD8+ T cell responses recognize HBV-infected hepatocytes. Targeting of these unique CD8+ T cell restriction molecules would constitute a new paradigm for the treatment of HBV infection and theoretically could be universally applied to all patients given the extreme conservation of MHC-E across humans and macaques (Wu *et al.* 2018. The Role of MHC-E in T Cell Immunity Is Conserved among Humans, Rhesus Macaques, and Cynomolgus Macaques. J. Immunol. 200: 49–60).

[0109] Four rhesus macaques (RM) were inoculated with strain 68-1 RhCMV expressing HBV genotype D serotype ayw core, surface, and/or polymerase antigens. Genotype D, serotype ayw HBV core, polymerase, and S Ag gene fragments were isolated by PCR from previously described plasmids (Frank Chisari, Scripps Research Institute). The N-terminal 333 amino acids of polymerase obtained from plasmid pCDNA3-POL/ENV (Kakimi, K. *et al.* 2002. Immunogenicity and tolerogenicity of hepatitis B virus structural and nonstructural proteins: implications for immunotherapy of persistent viral infections. J. Virol. 76: 8609–8620) were C-terminally HA-epitope tagged and fused by PCR-mediated mutagenesis to the C-terminal 228 amino acids of the S Ag obtained from

plasmid pCMV-S2/S (Michel, M. L., *et al.* 1995. DNA-mediated immunization to the hepatitis B surface antigen in mice: aspects of the humoral response mimic hepatitis B viral infection in humans. Proc. Natl. Acad. Sci. USA 92: 5307–5311.) to generate fusion S/PolN (left forward primer: 59-CATCGAGCTAGCACCATGGAGAACATCACATCAGG-39 (SEQ ID NO: 1), left reverse primer: 59-GTGTTGATAGGATAGGGGAATGTATACCCAAAGAC-39 (SEQ ID NO: 2); right forward primer: 59-GTCTTTGGGTATACATTCCCCTATCCTATCAACAC-39 (SEQ ID NO: 3), right reverse primer: 59-GGAATCGTCGACTCAAGCGTAATCTGGAACATCGTATGGGTAAAGATTGACG ATAAGGGAGAGGCAG-39 (SEQ ID NO: 4)). The final PCR product was blunt-end cloned into either pJet vector (Thermo Fisher Scientific) to be a template for bacterial artificial chromosome (BAC) recombineering or into pORI to evaluate expression. The C-terminal 416 amino acids of polymerase obtained from plasmid pCDNA3-POL/ENV (Kakimi, K. *et al.* 2002. Immunogenicity and tolerogenicity of hepatitis B virus structural and nonstructural proteins: implications for immunotherapy of persistent viral infections. J. Virol. 76: 8609–8620) was HA-epitope tagged by PCR-mediated mutagenesis and inserted into pORI (forward primer: 59-GTGGTACCCTCGAGGATTGGGGACCCTGCGCTGAACATGGAG-39 (SEQ ID NO: 5), reverse primer: 59-TCAGTCGACCTAAGCGTAATCTGGAACATCGTATGGGTAC-39 (SEQ ID NO: 6)). The gene encoding Core was PCR amplified from plasmid pCDNA-CORE (22) and inserted into pORI (forward primer: 59-CTGCTAGCATGGACATTGACCCTTATAAAGAATTTGG-39 (SEQ ID NO: 7), reverse primer: 59-CTAGGTACCACATTGAGATTCCCGAGATTGAG-39 (SEQ ID NO: 8)). The C-terminal polymerase fragment was then inserted downstream of Core using KpnI and SalI to generate fusion protein HBV core and the C terminus of polymerase (Core/PolC). The KpnI site adds a 2-amino acid (GT) linker between the two proteins. To generate 68-1 RhCMV/Core/PolC and 68-1 RhCMV/S/PolN, the pp71-encoding Rh110 gene in the 68-1 RhCMV BAC (Chang, W. L. W., *et al.* 2003. Cloning of the full-length rhesus cytomegalovirus genome as an infectious and self-excisable bacterial artificial chromosome for analysis of viral pathogenesis. J. Virol. 77: 5073–5083) was replaced using a modified galactokinase (galK) selection system, a two-step

method that allows DNA modification without introducing unwanted heterologous sequences (Warming, S. *et al.* 2005. Simple and highly efficient BAC recombineering using galK selection. Nucleic Acids Res. 33: e36). It was recently demonstrated that replacement of Rh110 can be used to elicit robust Ag responses while attenuating the 68-1 RhCMV vector (Marshall, E. E., *et al.* 2019. Enhancing safety of cytomegalovirus-based vaccine vectors by engaging host intrinsic immunity. Sci. Transl. Med. 11: eaaw2603 10.1126/scitranslmed.aaw2603).

[0110] To delete Rh110, competent SW105 bacteria containing the 68-1 RhCMV BAC were electroporated with a PCR product containing a galK/kanamycin cassette with 50-bp flanking homology to Rh110. The bacteria were plated on kanamycin/chloramphenicol Luria Bertani agar at 30°C for positive selection. To replace the galK/kanamycin cassette with the HBV fusion genes, a PCR product containing the HBV S–PolN fusion or HBV Core–PolC fusion with the same flanking homology to Rh110 was electroporated, and the bacteria were plated on 2-deoxy-galactose (DOG) chloramphenicol minimal media plates with glycerol as the carbon source for negative selection. PCR primers for homologous recombination were as follows: Rh110 S/PolN forward: 59-GATCACGTCATTGACACCGGCCTCCCACCAGCTCTCACATTCTCCGCATCACCATGGAGAACATCACATCAGGAT-39 (SEQ ID NO: 9), Rh110 S/PolN reverse: 59-CAAAATATTATTACATGGTACGCAATTTATTGTCTATTTTCGTTATTTGTTTATTCAAGCGTAATCTGGAACATCGTAT-39 (SEQ ID NO: 10) and Rh110 Core/PolC forward: 59-GATCACGTCATTGACACCGGCCTCCCACCAGCTCTCACATTCTCCGCATCACCATGGACATTGACCCTTATAAAGAAT-39 (SEQ ID NO: 11), Rh110 Core/PolC reverse: 59-CAAAATATTATTACATGGTACGCAATTTATTGTCTATTTTCGTTATTTGTTTATCTAAGCGTAATCTGGAACATCGTAT-39 (SEQ ID NO: 12). To generate 68-1 RhCMV/Core, we amplified the HBV core gene from pCDNA-CORE and introduced an N-terminal FLAG-tag by PCR (forward primer: 59-CTGCTAGCATGGATTACAAGGATGACAAGGACATCGACCCTTATAAAGAATTTGG-39 (SEQ ID NO: 13); reverse primer: 59-CTAGTCGACACATTGAGATTCCCGAGATTGAG-39) (SEQ ID NO: 14). The amplified product was cloned into pORI downstream of the EF1a promoter. This expression cassette was inserted into Rh211 region of 68-1 RhCMV together with a Kan

resistance cassette flanked by flippase recognition target sites by homologous recombination using primers containing 50-bp homology to regions of Rh211 (forward primer: 59-GGGAAATCACGTCATCAGGCTGGGTAGTCAACATGGGCATACGAAACTTGCCCGAATAGATGCTCTCACTTAACGGCTGACATG-39 (SEQ ID NO: 15), reverse primer: 59-CCAGAATGTGCTCTACTTTTTGGCCAGCGGGTTGGATGATTTCGCGCGTCATGGACTGCTTCACTGTAGCTTAGTACGTTAAAC-39 (SEQ ID NO: 16)). The PCR fragment was electroporated into EL250 bacteria containing the RhCMV 68-1 BAC for in vivo recombination and recombinants selected for Kan resistance. The Kan resistance cassette was removed by temperature-inducible flippase recombination. The resulting BACs were analyzed by restriction digest, PCR analysis of recombination sites, and next-generation sequencing on an Illumina MiSeq sequencer. This sequence analysis revealed two point mutations in S/PolNthat were introduced during PCR amplification resulting in amino acid exchanges A118T and T125M in the S Ag. BAC DNA was purified using alkaline lysis, phenol/chloroform extraction, and isopropanol precipitation, and virus was reconstituted by transfection of BAC DNA using Lipofectamine 2000 (following manufacturer's protocol; Thermo Fisher Scientific) of telomerized pp71 expressing rhesus fibroblasts (Warming, S. *et al.* 2005. Simple and highly efficient BAC recombineering using galK selection. Nucleic Acids Res. 33: e36) or primary rhesus fibroblasts. Expression of HBV Ags was confirmed by infecting telomerized RM fibroblasts with 68-1 RhCMV/Surface/PolN or RhCMV/Core/PolC. Cells were harvested at full cytopathic effect and lysed in SDS sample buffer. 293T cells transfected (Lipofectamine 2000) with the pORI expression plasmids containing the HA-tagged HBV proteins served as positive controls. After electrophoretic separation, immunoblots were performed with anti-HA Ab MMS-101P (Covance MMS).

[0111] Two RM (RM1, RM2) were inoculated with both 68-1 RhCMV/Surface/PolN and 68-1 RhCMV/Core/PolC vectors, and two additional RM (RM3, RM4) were innoculated with a 68-1 RhCMV–based vector that expressed HBV Core under the EF1a promoter. The CD8+ T cell response against each of the Ags was longitudinally monitored by ICS, using pools of overlapping 15-mer peptides corresponding to each Ag. Longitudinal CD8+ T cell responses against these antigens in the blood of vaccinated RM were observed (Fig. 1A).

[0112] The MHC-restriction of HBV core (HBcAg)-specific CD8+ T cell responses in these animals was characterized via intracellular cytokine staining with reagents that specifically block presentation by MHC-I, MHC-II, and MHC-E as previously described (Hansen *et al.* 2016. Broadly targeted $CD8^+$ T cell responses restricted by major histocompatibility complex E. Science 351: 714–720; Hansen, *et al.* 2013. Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. Science 340: 1237874–1237874.). It was found that, similar to strain 68-1 RhCMV vectors expressing SIV or *Mycobacterium tuberculosis* antigens, strain 68-1 RhCMV/HBV vector elicited HBV-specific, MHC-E- and MHC-II-restricted CD8+ T cell responses targeting a broad array of HBcAg peptides (Fig. 1B).

[0113] To further confirm that the MHC-E-restriction of the RhCMV/HBV engendered the CD8+ T cell responses observed, splenocytes from a RhCMV/HBV-vaccinated RM were stimulated with K562 cells (MHC-null) transduced to express either a single human (HLA) or rhesus macaque (Mamu) MHC-E allele and pulsed with one of three individual HBcAg 15-mer peptides identified as MHC-E restricted via blocking in Figure 1B. Only cells expressing MHC-E can present HBcAg 15-mers to these CD8+ T cells. The HBcAg-specific CD8+ T cells recognized their cognate antigen presented in the context of both HLA-E and Mamu-E (Fig. 1C). These results demonstrate the presence of MHC-E-restricted, HBV-specific CD8+ T cells in RhCMV/HBV-vaccinated RM, and further support the high functional conservation primate MHC-E molecules.

EXAMPLE 2: HBV-INFECTED PRIMARY HEPATOCYTES EXPRESS MHC-E IN VITRO

[0114] Next, it was determined if primary hepatocytes express MHC-E. Primary hepatocytes (PH) were isolated from three unrelated RM and three unrelated human donors (HD). To isolate RM primary hepatocytes, a single lobe of RM liver was perfused with 200 mL pre-perfusion media (0.5 mM EGTA (Bio-World, cat#:40120128-1), 10 IU/mL heparin (Fresenius Kabi, cat#:C504730), HBSS with calcium and magnesium (Fisher Scientific, cat#:24-020-117)), followed by 200 mL HBSS without calcium and magnesium (Fisher Scientific, cat#:SH3003103) to remove remaining EGTA. Next 100 mL of collagenase media (DMEM/F12 (Gibco, cat#:11320-082), 1mM calcium chloride (Sigma-Aldrich, cat#:C5670-100G), 20mM HEPES (HyClone, cat#:SH30237.01) 1mg/mL collagenase IV (Sigma-Aldrich, cat#:C9722-50MG)) warmed to 42°C was

perfused into the lobe and discarded. This was followed by re-circulation of 150 ml collagenase media through the liver lobe at 42°C for 30 minutes to 1 hour using a rate of 75-150 mL/min, depending on the size of the liver lobe. Following collagenase perfusion, the liver was filleted with scalpels, washed over with remaining collagenase media, and media was filtered through a tea strainer. PH were washed three times in wash media (DMEM/F12, 2% bovine growth serum (HyClone, cat#:SH3054103), 23 mM HEPES buffer, 0.6 mg/ml glucose, 2mM L-glutamine (HyClone, cat#:SH3003401), 1x antibiotic/antimycotic (HyClone, cat#:SV3007901), and 0.1 mg/mL Gentamicin (Life Technologies, cat#:15750-060)) at room temperature, with centrifugation between each wash at 50 x g for 3 minutes. Prior to the third wash spin, PH were passed through a 70 μM filter to ensure single-cell suspension. PH were then suspended in 20ml of 36% isotonic percoll (GE Healthcare, cat#:17-0891-01) in a 50ml conical using PH media as a diluent (DMEM/F12, 10% bovine growth serum, 23 mM HEPES buffer, 0.6 mg/ml glucose, 2mM L-glutamine, 1x antibiotic/antimycotic, and 0.1 mg/mL Gentamicin), and centrifuged at 200 x g for 7 minutes. The purified PH pellet was then resuspended in room temperature PH media and counted. Collagenized plates for the hepatocytes were prepared using 0.2 mg/mL collagen R in 0.01% acetic acid (Serva, cat#:47254), left on the plate for at least 20 min prior to washing with 1 mL HBSS immediately prior to plating at 2 x 10^5 PH per well in a 12-well plate. Plates were placed at 37°C, 5% CO2. The next day, wells were washed twice with HBSS and cultured in 1 ml PH media supplemented with 1.8% DMSO (primary hepatocyte media containing DMSO ; PH-DMSO) for the remainder of the experiment.

[0115] Human donor primary hepatocytes (HD PH) were isolated from murine humanized livers and purchased from Yecuris, Inc. Humanized mice were generated with cryopreserved primary hepatocytes collected from deceased patients with the following demographics: HD1 (13 year old, female, Hispanic, HBV naïve); HD2 (13 year old, female, Caucasian, HBV naïve); HD3 (27 year old, male, Caucasian, HBV naïve).

[0116] RM and HD PH MHC presentation was determined by surface expression of bulk MHC-I via the W6/32 clone, MHC-II via HLA-DR staining, and MHC-E via the 4D12 clone. It was previously demonstrated that 4D12 specifically stains Mamu-E and not classical Mamu-Ia molecules. All three human donors shared one HLA-A and one HLA-C allele (Table 1). Thus, before proceeding it was confirmed that the MHC-E-specific

4D12 clone stains only HLA-E and not the HLA-A or -C molecules shared between the three HD (Figure 2A). Expression of MHC-E was examined by staining and majority of primary hepatocytes from both species expressed MHC-E (Figs. 2B and 2C).

Table 1. MHC Genotypes of HD PH

	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C
HD1	02:17	02:01	40:02	50:01	03:05	06:02
HD2	01:01	02:01	15:XX*	37:XX*	03:03	06:02
HD3	02:01	02:01	57:01	27:05	02:02	06:02

XX* denotes an undetermined allele.

[0117] To determine if HBV infection influences the expression of MHC-E on the surface of primary hepatocytes, primary hepatocytes were collected from the same HBV infected human donor. One day after plating isolated rhesus macaque primary hepatocytes, replication-incompetent adenovirus serotype 5 expressing human NTCP (MOI 10) under the liver-specific TTR promoter was added to the culture for 2 days. On the second day, cells were re-fed with 1ml PH-DMSO media. On the fourth day following adenovirus transduction, primary hepatocytes were washed twice in 1 ml HBSS and overlaid with HBV-containing media at an MOI of 100 (PH-DMSO containing 4% PEG6000, Sigma-Aldrich, cat#:81253-250G) and incubated overnight. The next morning, wells were washed three times with 1 ml HBSS and then cultured in 1 ml primary hepatocytes -DMSO for the remainder of the experiment.

[0118] One day after plating, human donor primary hepatocytes were overlaid with HBV-containing media at an MOI of 100 (PH-DMSO containing 4% PEG6000, Sigma-Aldrich, cat#:81253-250G) and incubated overnight. The next morning, wells were washed three times with 1 ml HBSS and then cultured in 1 ml primary hepatocytes -DMSO for the remainder of the experiment.

[0119] HBV infection of human donor primary hepatocytes was confirmed by measuring the level of HBV envelope antigen (HBeAg) in the supernatant prior to staining of the cells. Primary hepatocytes were co-stained with MHC markers (MHC-I, MHC-E, and MHC-II) along with intracellular HBcAg on day 4 post-HBV infection, since this was the first time point where intracellular HBcAg was detectable. Strong staining with the 4D12 antibody was observed on both HBV-infected and HBV-naïve PH, indicating high levels of MHC-E expression (Figs. 2D and 2E). In contrast, HLA-DR expression was minimal

or absent in all three human donor primary hepatocytes samples, in line with previously published results (Senaldi *et al.* 1991. Class I and class II major histocompatibility complex antigens on hepatocytes: importance of the method of detection and expression in histologically normal and diseased livers. J. Clin. Pathol. 44: 107–114.). It is possible that *ex vivo* manipulation of the primary hepatocytes prior to assessing surface MHC levels induced a fraction of these cells to lose MHC-E expression. Nevertheless, taken together, these results showed that HBV-infected primary hepatocytes express MHC-E and that MHC-E could represent a potential HBV-specific CD8+ T cell restriction element.

EXAMPLE 3: MHC-E-RESTRICTED CD8+ T CELLS FROM RHCMV/HBV-INOCULATED RHESUS MACAQUES RECOGNIZE HBV-INFECTED ALLOGENIC AND XENOGENIC PRIMARY HEPATOCYTES

[0120] The MHC study revealed high levels of MHC-E expression on human donor and rhesus macaque primary hepatocytes, regardless of HBV infection. It was therefore hypothesized that CD8+ T cells from RhCMV/HBV inoculated rhesus macaques would recognize allogeneic, HBV-infected rhesus macaque primary hepatocytes given the high functional conservation of MHC-E in primates. In support of this hypothesis, CD8+ T cells (bulk splenocytes and purified CD8β+ T cells) from RM1 and RM2 recognized HBV-infected primary hepatocytes from two unrelated rhesus macaque donors, but did not respond to HBV-naïve primary hepatocytes (Fig. 3A).

[0121] In order to more comprehensively determine the MHC-restriction of CD8+ T cells recognizing HBV-infected primary hepatocytes targets, a series of recognition experiments were performed using MHC-specific blocking reagents shown in Fig. 1.

[0122] Prior to HBV infection, one well of primary hepatocytes was collected and stained as a baseline. Starting on day two post-infection, a well each of HBV-infected and HBV-naïve primary hepatocytes was collected with 0.5% trypsin-EDTA (Fisher Scientific, cat#: SH30236.01) and washed twice in ice cold FACS buffer (PBS, Fisher Scientific, cat#:SH30256FS, with 10% fetal bovine serum). Cells were incubated with anti-HLA-E antibody (clone: 4D12, Origene, cat#: LS-C179742) for 30 minutes at 4°C, washed twice in ice cold FACS buffer, and incubated with F(ab)2-Goat anti-mouse IgG (H L)-APC (Invitrogen, cat#: A10539) for 30 minutes at 4°C. Cells were then washed twice in ice cold PBS and incubated with pan-MHC-I-PerCP-Cy5.5 (clone: W6/32,Biolegend Inc.,

cat#: 311419), anti-HLA-DR Alexa 700 (clone: L243 (BD Biosciences, cat#: 560743), and Live/Dead fixable yellow (Invitrogen, cat#: L-34959) for 30 minutes at 4°C. Cells were washed in FACS buffer and fixed using Foxp3 / Transcription Factor Staining Buffer Set (eBioscience, cat#: 00-5523-00) for one hour at room temperature. Prior to fixation all wash spins were performed at 350 x g for 3 minutes. After fixation, cells were suspended in permeabilization buffer (eBioscience, cat#: 00-8333-56). All wash spins after fixation were performed at 830 x g for 3 minutes. Primary hepatocytes were incubated for one hour at 4°C with Hepatitis B Virus Core Antigen Antibody (clone: 13A9, Fisher Scientific, cat#: MA1-7606) conjugated to R-phycoerythrin (PE) using the Lightning-Link R-PE kit (Innova Biosciences, cat#: 703-0005). Cells were washed three times in permeabilization buffer and then collected on a Becton-Dickenson LSR-II. Analysis was performed on FlowJo X (TreeStar Inc.). In all analyses, gating on the light scatter signature of large, complex PH was followed by assessment of specific MHC and HBV markers.

[0123] HBV-specific CD8+ T cell responses were measured in mononuclear cell preparations from the spleens of RhCMV/HBV vaccinated RM by flow cytometric ICS. Briefly, splenocytes or isolated CD8β+ T cells were incubated with HBV-infected or HBV naïve primary hepatocytes targets and the co-stimulatory molecules CD28 and CD49d (BD Biosciences) for 1 hour, followed by addition of brefeldin A (Sigma-Aldrich) for an additional 8 hours. Co-stimulation without primary hepatocytes target co-culture served as a background control. The MHC-restriction of a response was determined by pre-incubating PH targets for 1 hour at room temperature in the presence of pan anti-MHC-I antibody (25 μg/ml; clone: W6-32), VL9 peptide (20uM), CLIP peptide (MHC-II–associated in- variant chain, amino acids 89 to 100; 10 μg/ml), or anti-HLA-DR antibody (10μg/ml; clone: L243) before co-culturing with target primary hepatocytes cells. Stimulated cells were fixed, permeabilized, and stained, and flow cytometric analysis was performed on an LSR-II instrument (BD Biosciences). Analysis was done using FlowJo X software (Tree Star, Inc.). In all analyses, gating on the light scatter signature of small lymphocytes was followed by progressive gating on the CD3+ population and then the CD4–/CD8+ T cell subset. Antigen-specific response frequencies for CD8+ T cell populations were routinely determined by intracellular expression of IFN-γ.

[0124] HBV-infected or HBV-naïve targets were collected at day 6 post-HBV infection (MOI = 100), incubated with the blocking agents W6/32 antibody (pan MHC-I), VL9 peptide (MHC-E), CLIP (MHC-II), or HLA-DR antibody (MHC-II), and then co-cultured with splenocytes or isolated CD8β+ T cells overnight. Following co-culture, CD8+ T cells were stained intracellularly for IFN-γ and TNF-α to assess recognition of the targets. CD8+ T cell recognition of HBV-infected RM PH was blocked with W6/32 antibody and VL9 peptide, but not with CLIP or HLA-DR, indicating that the entirety of the response to HBV-infected targets was MHC-E restricted (Fig. 3A).

[0125] Because MHC-E is functionally conserved across primates, it was hypothesized that CD8+ T cells from RhCMV/HBV inoculated RM would also recognize HBV-infected HD primary hepatocytes. To test this hypothesis, similar recognition experiments were performed by incubating splenocytes and purified CD8+ T cells from the same RhCMV/HBV inoculated macaques (RM1 and RM2) with HBV-infected human donor primary hepatocytes. As hypothesized, these CD8+ T cells recognized HBV-infected, xenogeneic human donor primary hepatocytes (Fig. 3B). As described above for the rhesus macaque primary hepatocytes target co-culture experiments, the CD8+ T cell recognition of HBV-infected human donor primary hepatocytes was completely blocked by the MHC-E-binding VL9 peptide. Taken together, these results definitively show that HBV-infected primary hepatocytes present HBV antigen in the context MHC-E, indicating that this pathway can be exploited to target CD8+ T cells to HBV-infected cells.

[0126] Next, the conservation of two 15-mer peptides, Core 7 (PSVRDLLDTASALYR; SEQ ID NO: 17) and Core 14 (TALRQAILCWGELMT; SEQ ID NO: 18) MHC-E-bound supertope in the HBV core antigen was examined. 6,203 full genome HBV sequences spanning all known HBV genotypes were retrieved from The Hepatitis B Virus Database, translated, and amino acid sequences aligned against Core 7 (Fig. 4A) and Core 14 (Fig. 4B). There was high conservation within the two 15-mer peptides that generated MHC-E-restricted CD8+ T cell responses in all animals assayed (supertopes). Importantly, in the two positions not highly conserved (position 3 Core 7 and position 15 Core 14), there is only one additional dominant amino acid globally amongst HBV strains at this position. Thus, over 98% of known global sequences express one of two amino acids at these positions.

[0127] These results identify a completely new set of CD8+ T cell responses against HBV, which paves the way for development of innovative HBV therapeutics. While MHC-E-restricted CD8+ T cell responses have been identified in natural viral infections with CMV, EBV, and HCV (Joosten *et al.* 2016. Characteristics of HLA-E Restricted T-Cell Responses and Their Role in Infectious Diseases. Journal of Immunology Research 2016: 1–11), no reports of MHC-E-restricted CD8+ T cells responses against HBV have been published. Therefore, it was unclear whether HBV-infected hepatocytes presented HBV antigens in the context of MHC-E. These results show that MHC-E does present HBV antigens on the surface of HBV-infected cells and that CD8+ T cells from a completely distinct primate species can recognize these MHC-E:peptide complexes.

[0128] Outside of representing a completely unique type of CD8+ T cells response against HBV, the breadth of epitopes targeted within HBcAg indicates that therapeutic vaccination with CMV/HBV vectors would elicit broadly-targeted CD8+ T cell responses. While this broad targeting has been shown previously against SIV, *Mycobacterium tuberculosis*, and malaria, it may be particularly efficacious against HBV, since the vast majority of the HBV genome is comprised almost exclusively of non-plastic overlapping reading frames. Together, these results show for the first time that MHC-E-restricted CD8+ T cells can be harnessed for the treatment of chronic HBV infection, either through therapeutic vaccination or adoptive immunotherapy.

WHAT IS CLAIMED IS:

1. A method of generating an immune response to a hepatitis B virus (HBV) in a subject, the method comprising administering to the subject a CMV vector expressing a HBV antigen in an amount effective to elicit a CD8+ T cell response to the HBV antigen, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

2. A method of treating chronic HBV infection in a subject, the method comprising administering to the subject a CMV vector expressing a HBV antigen in an amount effective to elicit a CD8+ T cell response to the HBV antigen, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

3. A CMV vector expressing a HBV antigen for use in generating an immune response to a HBV in a subject, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

4. A CMV vector expressing a HBV antigen for use in the treatment of a chronic HBV infection in a subject, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

5. Use of a CMV vector expressing a HBV antigen in the manufacture of a medicament for use in generating an immune response to a HBV in a subject, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

6. Use of a CMV vector expressing a HBV antigen in the manufacture of a medicament for the treatment of a chronic HBV infection, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

7. The method, CMV vector for use, or use of a CMV vector in manufacture of any one of claims 1-6, wherein the hepatitis B virus antigen is a hepatitis B virus core, envelope, surface, X, or polymerase antigen.

8. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 7, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

9. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 8, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17).

10. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 8, wherein the hepatitis B antigen is TALRQAILCWGELMT (SEQ ID NO: 18).

11. The method, CMV vector for use, or use of a CMV vector in manufacture of any one of claims 1-10, wherein at least 10% of the CD8+ T cells elicited by the CMV vector are restricted by MHC-E or an ortholog thereof, or MHC-II or an ortholog thereof.

12. The method, CMV vector for use, or use of a CMV vector in manufacture of claim11, wherein at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, or at least 75% of the CD8+ T cells elicited by the CMV vector are restricted by MHC-E or an ortholog thereof, or MHC-II or an ortholog thereof.

13. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 11, wherein the CD8+ T cells restricted by MHC-E recognize an MHC-E supertope.

14. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 13, wherein the MHC-E supertope is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

15. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 13, wherein the MHC-E supertope is PSVRDLLDTASALYR (SEQ ID NO: 17).

16. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 13, wherein the MHC-E supertope is TALRQAILCWGELMT (SEQ ID NO: 18).

17. The method, CMV vector for use, or use of a CMV vector in manufacture of any one of claims 1-16, wherein fewer than 10% of the CD8+ T cells elicited by the CMV vector are restricted by MHC-class la or an ortholog thereof.

18. The method, CMV vector for use, or use of a CMV vector in manufacture of any one of claims 1-16, wherein some of the CD8+ T cells restricted by MHC-E recognize epitopes shared by at least 90% of other subjects immunized with the vector.

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

(a) administering to a first subject a recombinant CMV vector comprising a nucleic acid that expresses a HBV antigen, in an amount effective to generate a set of CD8+ T cells that recognize MHC-E/peptide complexes, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof;

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

(c) isolating one or more CD8+ T cells from a second subject; and

(d) 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 CD8+ T cells that recognize a MHC-E/HBV antigen peptide complex.

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

(a) isolating from a first subject a first set of CD8+ T cells, wherein the first subject has been administered a recombinant CMV vector comprising a nucleic acid that expresses a HBV antigen, in an amount effective to generate a set of CD8+ T cells that recognize MHC-E/peptide complexes, wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof;

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

(c) isolating a second set of CD8+ T cells from a second subject; and

(d) transfecting the second set of 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 CD8+ T cells that recognize a MHC-E/HBV antigen peptide complex.

21. The method of claim 19 or claim 20, wherein the recombinant CMV vector is a recombinant human CMV vector or a recombinant rhesus macaque CMV vector.

22. The method of any one of claims 19-21, wherein the hepatitis B virus antigen is a hepatitis B virus core, envelope, surface, or polymerase antigen.

23. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 22, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

24. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 23, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17).

25. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 23, wherein the hepatitis B antigen is TALRQAILCWGELMT (SEQ ID NO: 18).

26. The method of any one of claims 19-25, wherein the first set of CD8+ T cells recognizes specific hepatitis B virus antigen is a hepatitis B virus core, envelope, surface, X, or polymerase antigen peptides supertopes shared by at least 90% of other subjects immunized with the vector.

27. The method of claim 26, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

28. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 26, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17).

29. The method, CMV vector for use, or use of a CMV vector in manufacture of claim 26, wherein the hepatitis B antigen is TALRQAILCWGELMT (SEQ ID NO: 18).

30. The method of any one of claims 19-29, wherein the second set of CD8+ T cells recognizes hepatitis B virus antigen supertopes shared by at least 90% of other subjects immunized with the vector.

31. The method of claim 30, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

32. The method of claim 31, wherein the hepatitis B antigen is PSVRDLLDTASALYR (SEQ ID NO: 17).

33. The method claim 31, wherein the hepatitis B antigen is TALRQAILCWGELMT (SEQ ID NO: 18).

34. The method of any one of claims 19-33, wherein the first CD8+ TCR is identified by DNA or RNA sequencing.

35. The method of any one of claims 19-34, wherein the nucleic acid sequence encoding the second CD8+ TCR is identical to the nucleic acid sequence encoding the first CD8+ TCR.

36. The method of any one of claims 19-35, wherein the first subject and/or the second subject is a human or nonhuman primate.

37. The method of any one of claims 19-36, wherein the first subject is a nonhuman primate and the second subject is a human, and wherein the second CD8+ TCR is a chimeric nonhuman primate-human CD8+ TCR comprising the non-human primate CDR3α and CDR3β of the first CD8+ TCR.

38. The method of any one of claims 19-37 wherein the second CD8+ TCR comprises the non-human primate CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the first CD8+ TCR.

39. The method of any one of claims 19-38, wherein the second CD8+ TCR comprises CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the first CD8+ TCR.

40. The method of any one of claims 19-39, wherein the nucleic acid sequence encoding the second CD8+ TCR is identical to the nucleic acid sequence encoding the first CD8+ TCR.

41. The method of any one of claims 19-40, wherein the second CD8+ TCR is a chimeric CD8+ TCR.

42. The method of any one of claims 19-41, wherein the second CD8+ TCR comprises CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the first CD8+ TCR.

43. The method of any one of claims 19-42, wherein administering the CMV vector to the first subject comprises intravenous, intramuscular, intraperitoneal, or oral administration of the CMV vector to the first subject.

44. The method of any one of claims 19-43, further comprising administering the transfected CD8+ T cells to the second subject to treat or prevent HBV infection.

45. A CD8+ T cell generated by the method of any one of claims 19-44.

46. A method of treating or preventing a hepatitis B infection in a subject in need thereof, the method comprising administering the CD8+ T cell of claim 45 to the subject.

47. A CD8+ T cell of claim 45 for use in a method of treating or preventing a hepatitis B infection in a subject in need thereof.

48. Use of the CD8+ T cell of claim 45 in the manufacture of a medicament for use in a method of treating or preventing a hepatitis B infection in a subject in need thereof.

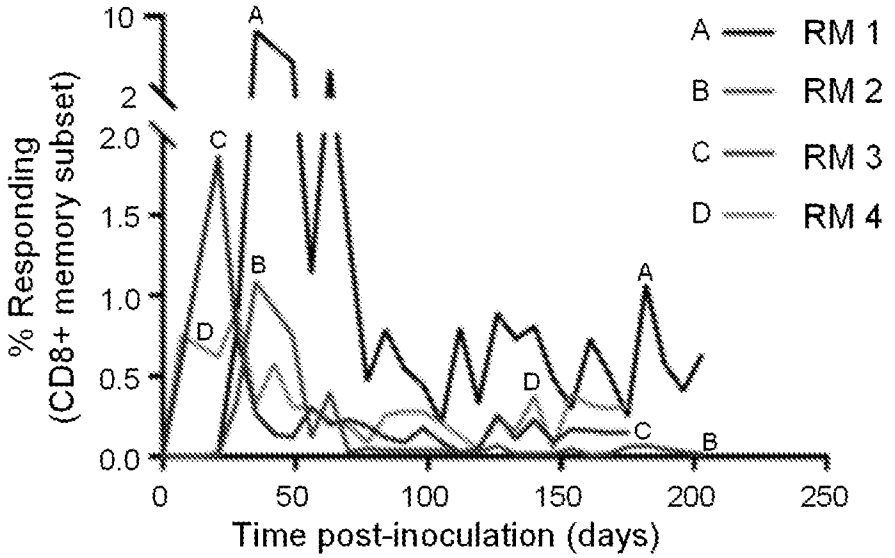
% Responding
(CD8+ memory subset)
10
2
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
250
Time post-inoculation (days)
A
B
C
D
A RM 1
B RM 2
C RM 3
D RM 4

FIG. 1A

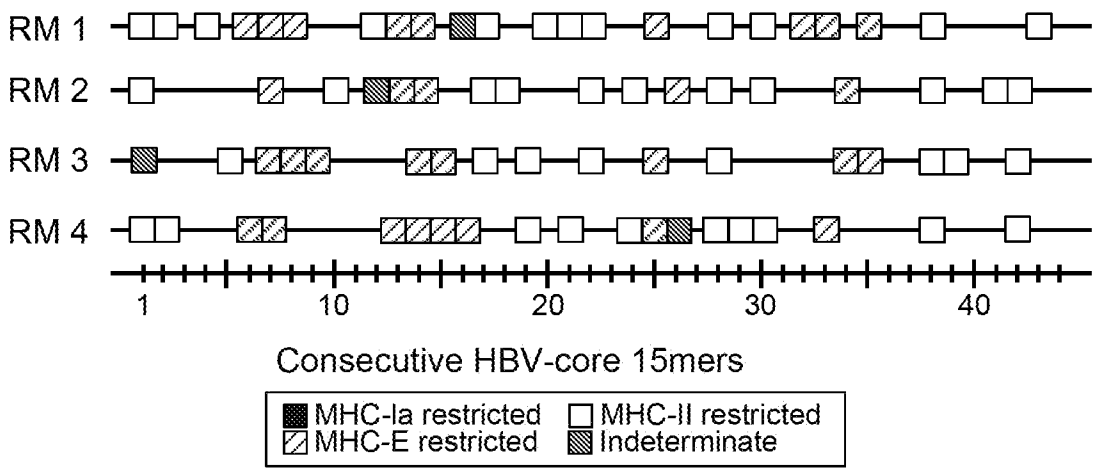
RM 1
RM 2
RM 3
RM 4
1
10
20
30
40
Consecutive HBV-core 15mers
MHC-Ia restricted
MHC-E restricted
MHC-II restricted
Indeterminate

FIG. 1B

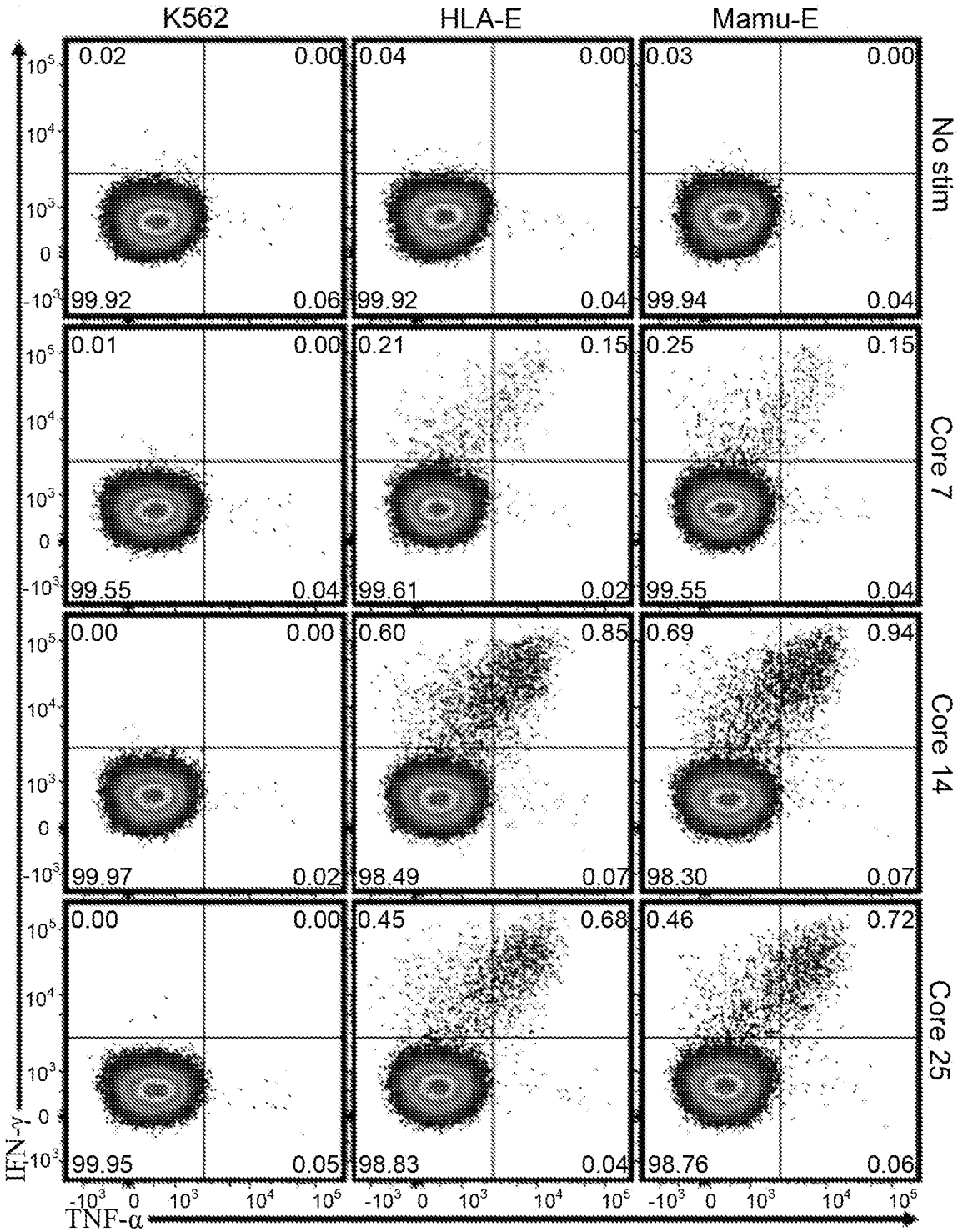
K562
HLA-E
Mamu-E
No stim
Core 7
Core 14
Core 25
IFN-γ
TNF-α
0.02
0.00
99.92
0.06
0.04
0.00
99.92
0.04
0.03
0.00
99.94
0.04
0.01
0.00
99.55
0.04
0.21
0.15
99.61
0.02
0.25
0.15
99.55
0.04
0.00
0.00
99.97
0.02
0.60
0.85
98.49
0.07
0.69
0.94
98.30
0.07
0.00
0.00
99.95
0.05
0.45
0.68
98.83
0.04
0.46
0.72
98.76
0.06

FIG. 1C

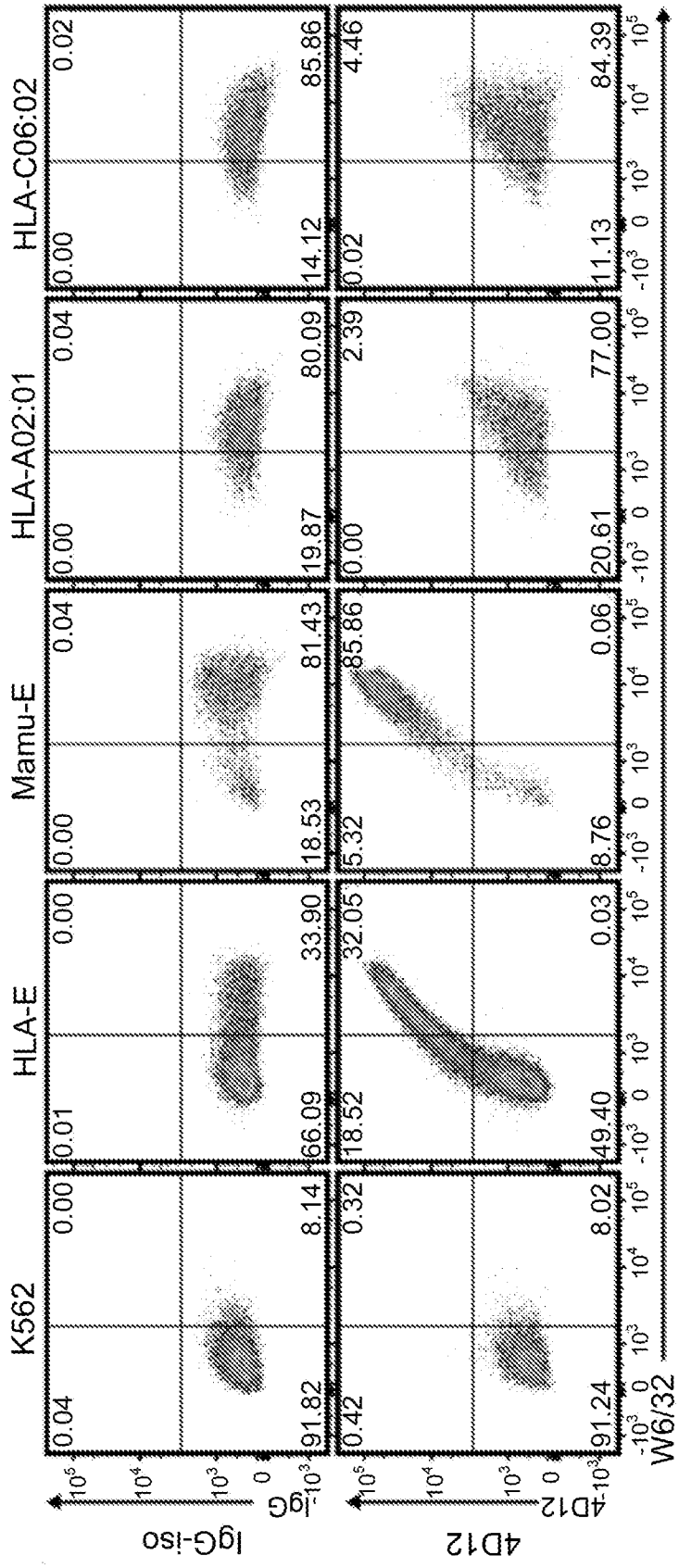
K562
HLA-E
Mamu-E
HLA-A02:01
HLA-C06:02
0.04
0.00
0.01
0.00
0.04
0.00
0.04
0.00
0.02
91.82
8.14
66.09
33.90
18.53
81.43
19.87
80.09
14.12
85.86
0.42
0.32
18.52
32.05
5.32
85.86
0.00
2.39
0.02
4.46
91.24
8.02
49.40
0.03
8.76
0.06
20.61
77.00
11.13
84.39
IgG-iso
4D12
W6/32

FIG. 2A

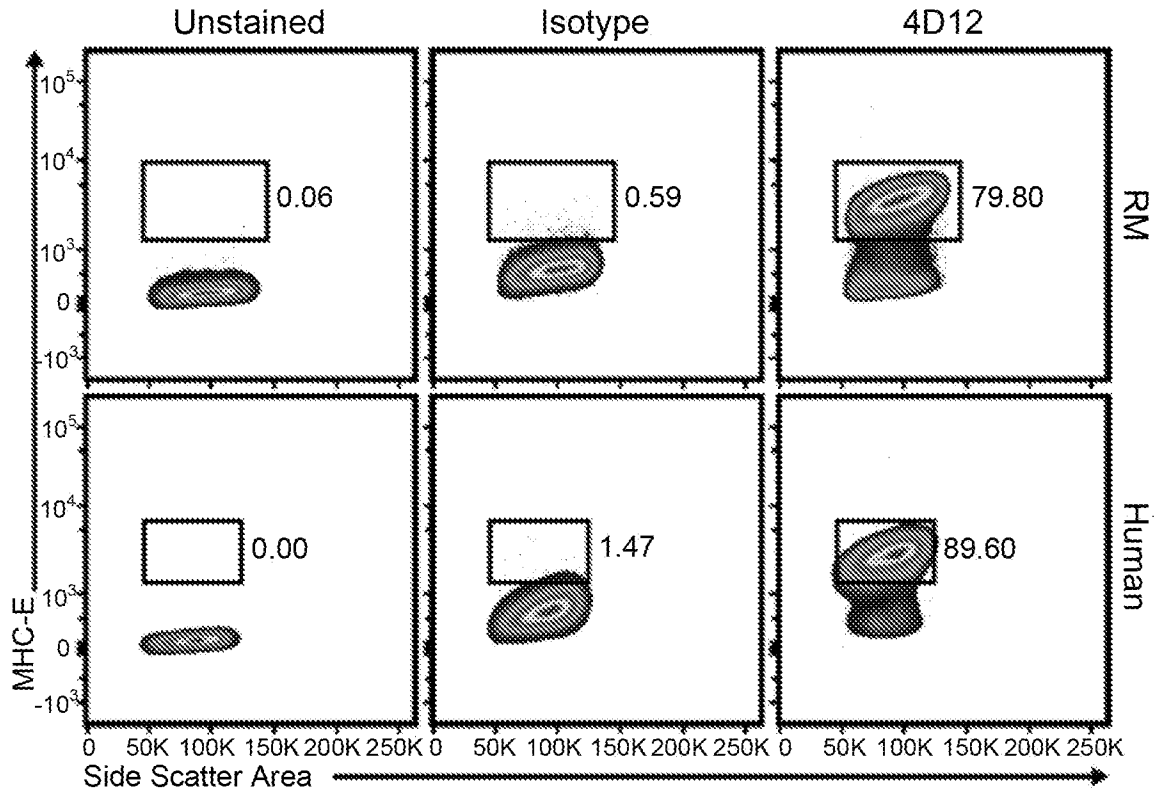
Unstained
Isotype
4D12
RM
Human
MHC-E
0.06
0.59
79.80
0.00
1.47
89.60
Side Scatter Area

FIG. 2B

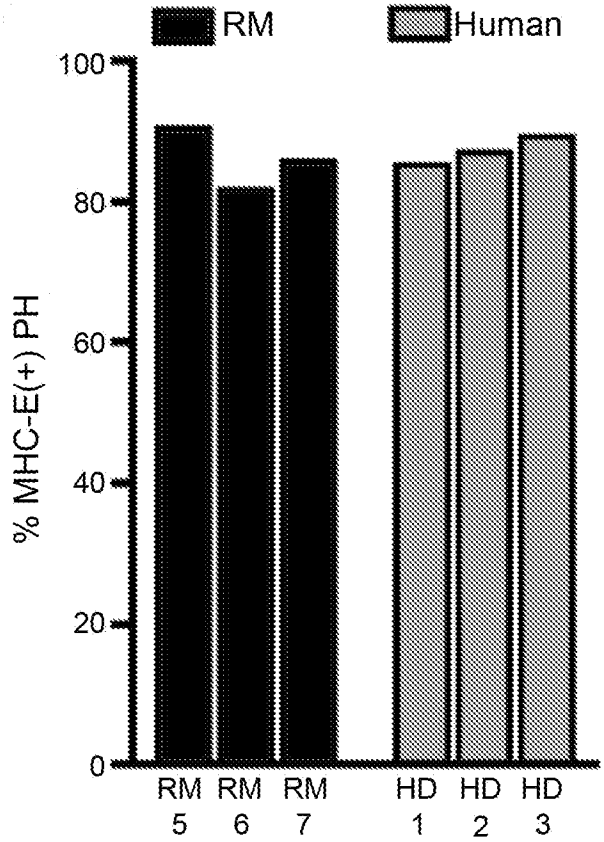
RM
Human
% MHC-E(+) PH
RM 5
RM 6
RM 7
HD 1
HD 2
HD 3

FIG. 2C

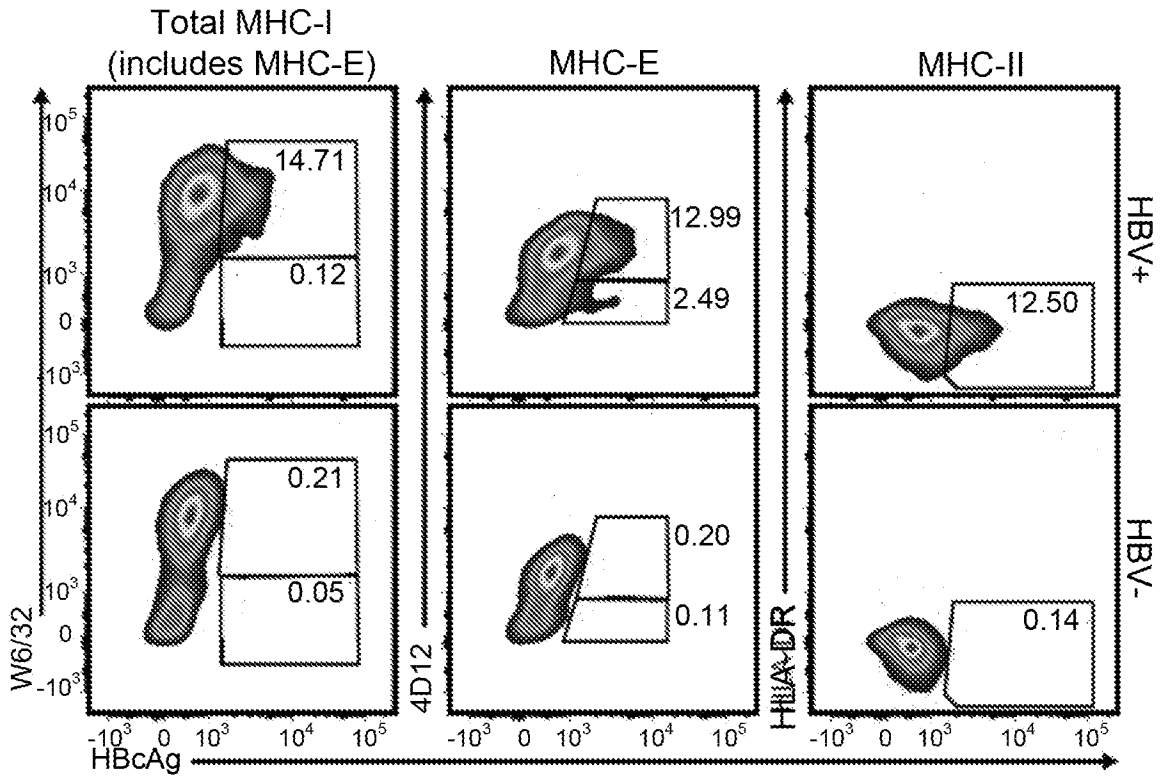
Total MHC-I
(includes MHC-E)
MHC-E
MHC-II
HBV+
HBV-
14.71
0.12
12.99
2.49
12.50
0.21
0.05
0.20
0.11
0.14
W6/32
4D12
HLA-DR
HBcAg

FIG. 2D

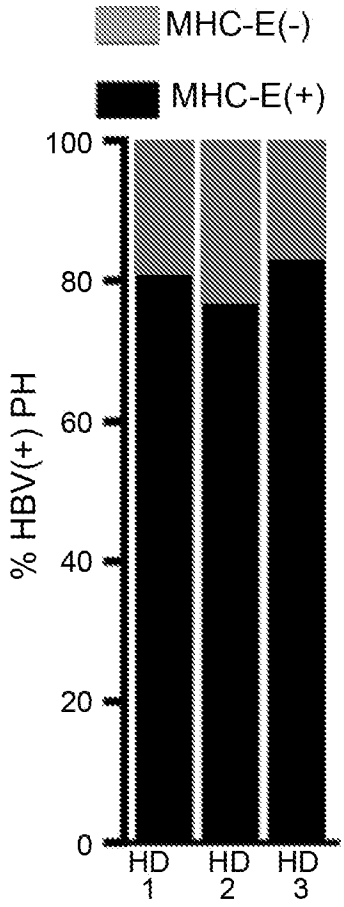
MHC-E(-)
MHC-E(+)
% HBV(+) PH
HD 1
HD 2
HD 3

FIG. 2E

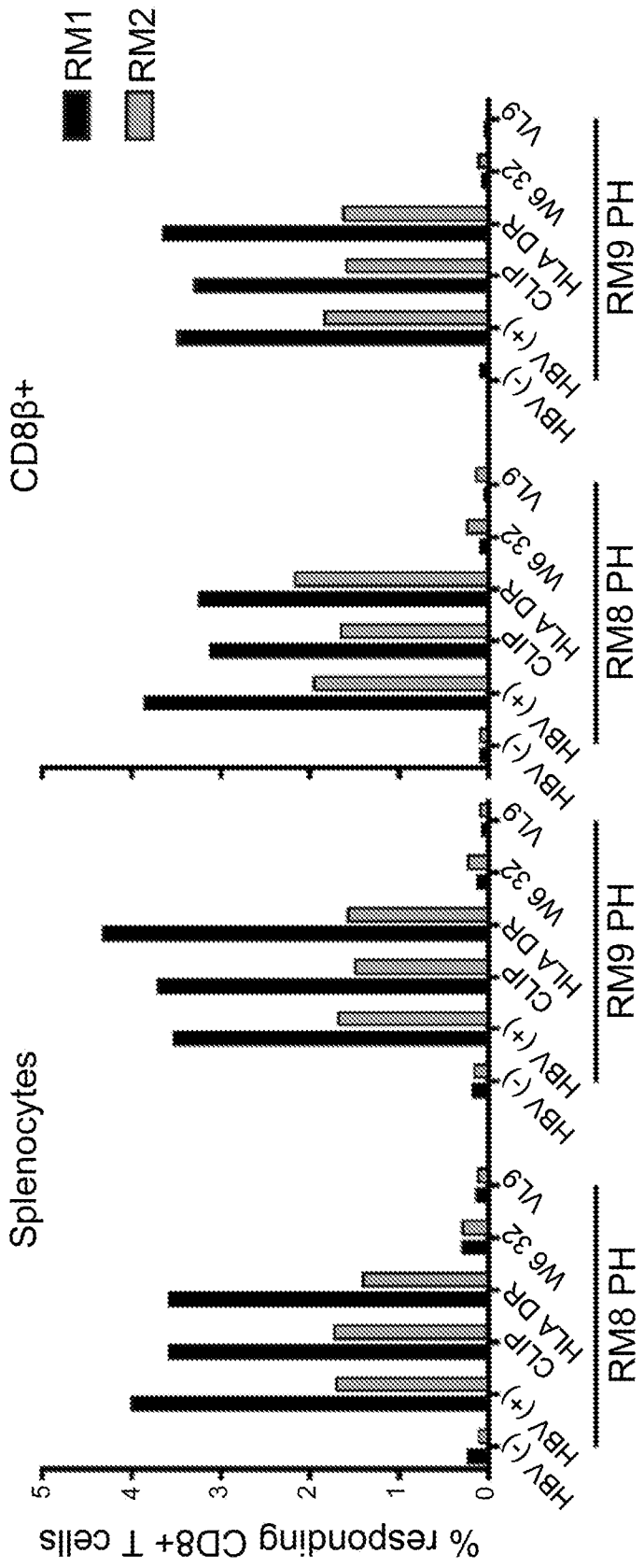
RM1
RM2
CD8β+
Splenocytes
% responding CD8+ T cells
0
1
2
3
4
5
HBV (-)
HBV (+)
CLIP
HLA DR
W6 32
VL9
RM8 PH
RM9 PH
RM8 PH
RM9 PH

FIG. 3A

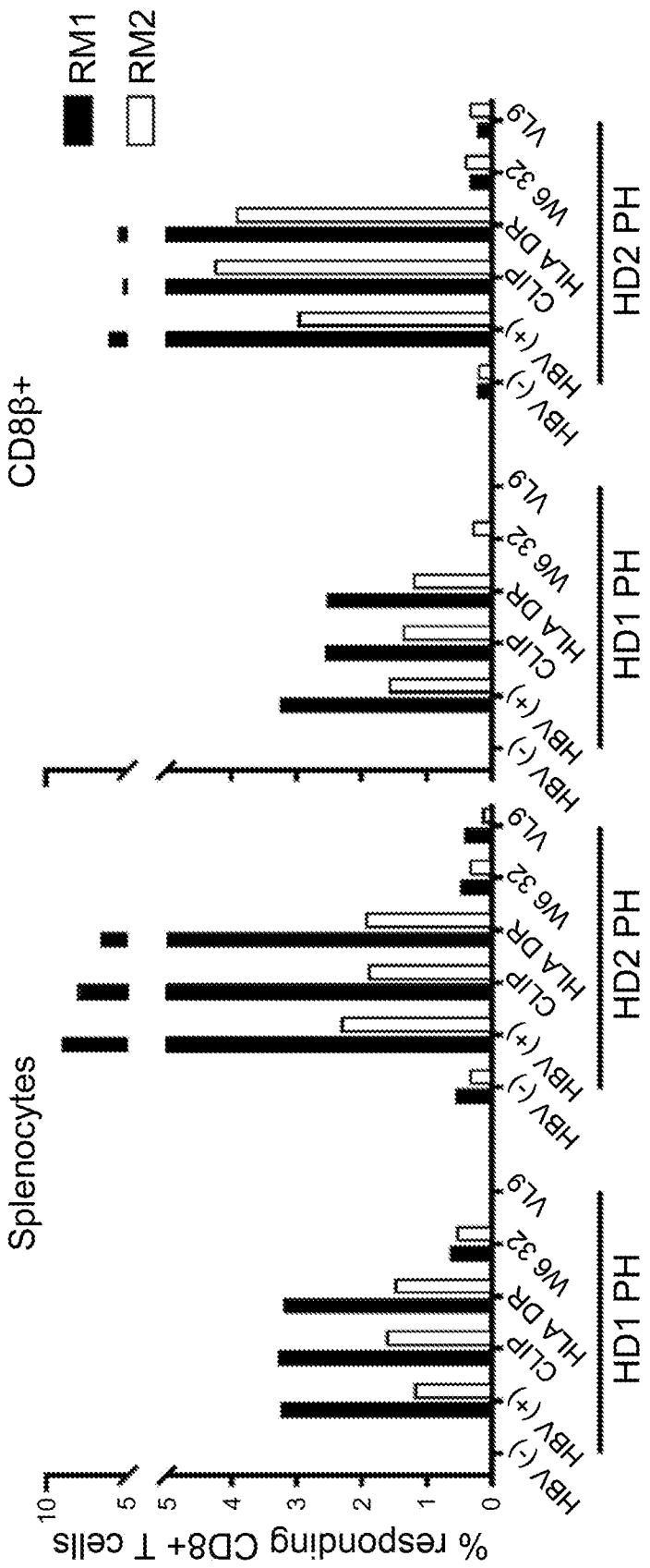
RM1
RM2
CD8β+
Splenocytes
% responding CD8+ T cells
0
1
2
3
4
5
10
HBV (-)
HBV (+)
CLIP
HLA DR
W6 32
VL9
HD1 PH
HD2 PH

FIG. 3B

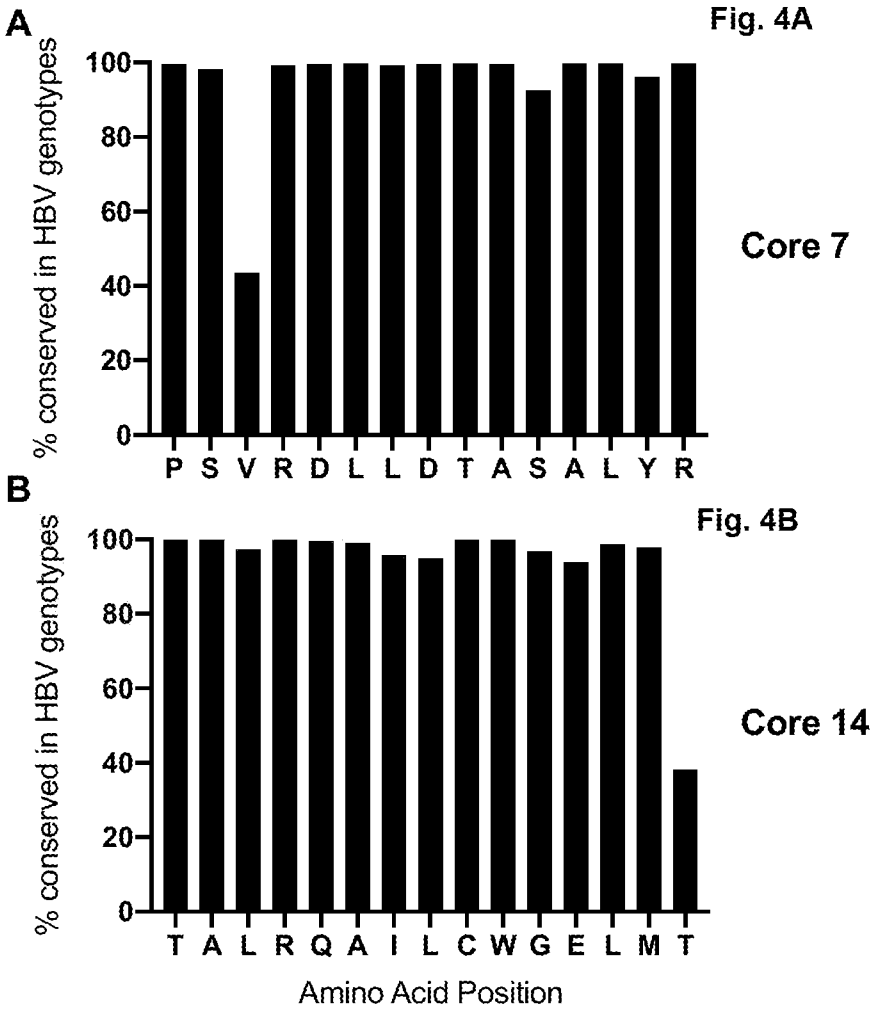

Fig. 4A

Core 7

Fig. 4B

Core 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/36480

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/86; C12N 15/869; C07K 7/08; C07K 14/03; A61K 39/12; A61K 39/29 (2020.01)

CPC - C12N 15/86; C12N 15/869; C07K 7/08; C07K 14/03; A61K 39/12; A61K 39/292; A61K 2039/5256; A61K 2039/572

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2018/0133321 A1 (Oregon Health & Science University) 17 May 2018 (17.05.2018). Especially para [0006], [0008], [0009], [0010], [0011], [0012], [0096].	19-21 --- 1, 2, 5, 6, (7-10)/(1,2,5,6)
Y	US 2007/0055049 A1 (Grey et al.) 8 March 2007 (08.03.2007). Especially para [0089], [0113], SEQ ID NO: 7524.	1, 2, 5, 6, (7-10)/(1,2,5,6)
Y	US 2006/0020110 A1 (Sallberg) 26 January 2006 (26.01.2006). Especially SEQ ID NO: SEQ ID NO: 97	9/(1,2,5,6)

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 2020	04 NOV 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/36480

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 11-18, 22-48
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-----Go to Extra Sheet for continuation-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 2, 5-6, (7-10)(in part), 19-21

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US 20/36480

Continuation of Box III: Observations where Unity of Invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1, 2, 5-6, (7-10)(in part), 19-21, drawn to administering or use of a CMV vector expressing a HBV antigen.

Group II: Claims 3, 4, (7-10)(in part), drawn to a CMV expression vector encoding a HBV antigen.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I has the special technical feature of administering or use of a CMV vector expressing a HBV antigen, not required by Group II.

Group II has the special technical feature of a composition comprising a CMV expression vector encoding a HBV antigen, not required by Group I.

Common Technical Features:

1. Groups I and II share the common technical feature of a CMV expression vector wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof.

2. Groups I and II share the common technical feature of a HBV antigen comprising PSVRDLLDTASALYR (SEQ ID NO: 17) or TALRQAILCWGELMT (SEQ ID NO: 18).

However, said common technical features do not represent a contribution over the prior art, and are disclosed by US 2018/0133321 A1 to Oregon Health & Science University (hereinafter "OHSU"), in view of US 2007/0055049 A1 to Grey et al. (hereinafter "Grey").

As to common technical feature #1, OHSU discloses a CMV expression vector wherein the CMV vector does not express an active UL128, UL130, UL146, and UL147 protein or orthologs thereof [that expresses a HBV antigen] (para [0006]; "Disclosed herein are CMV vectors comprising a first 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 orthologs thereof"; para [0010]; "the heterologous antigen of the CMV vectors disclosed herein may be any heterologous antigen, including a pathogen-specific antigen derived from, for example?..Hepatitis B Virus").

As to common technical feature #2, Grey discloses an HBV antigen comprising SEQ ID NO: 18: 1-TALRQAILCWGELMT-15 (SEQ ID NO: 7524; 17AA 1-TALRQAILCWGELMT-15 100% sequence identity; para [0113]; "Examples of suitable antigens particularly include hepatitis B core and surface antigens (HBVc, HBVs)").

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (cont.): Claims 11-18, 22-48 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).