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(54) **GENERATION OF BROADLY-SPECIFIC, VIRUS-IMMUNE CELLS TARGETING MULTIPLE HIV ANTIGENS FOR PREVENTIVE AND THERAPEUTIC USE**

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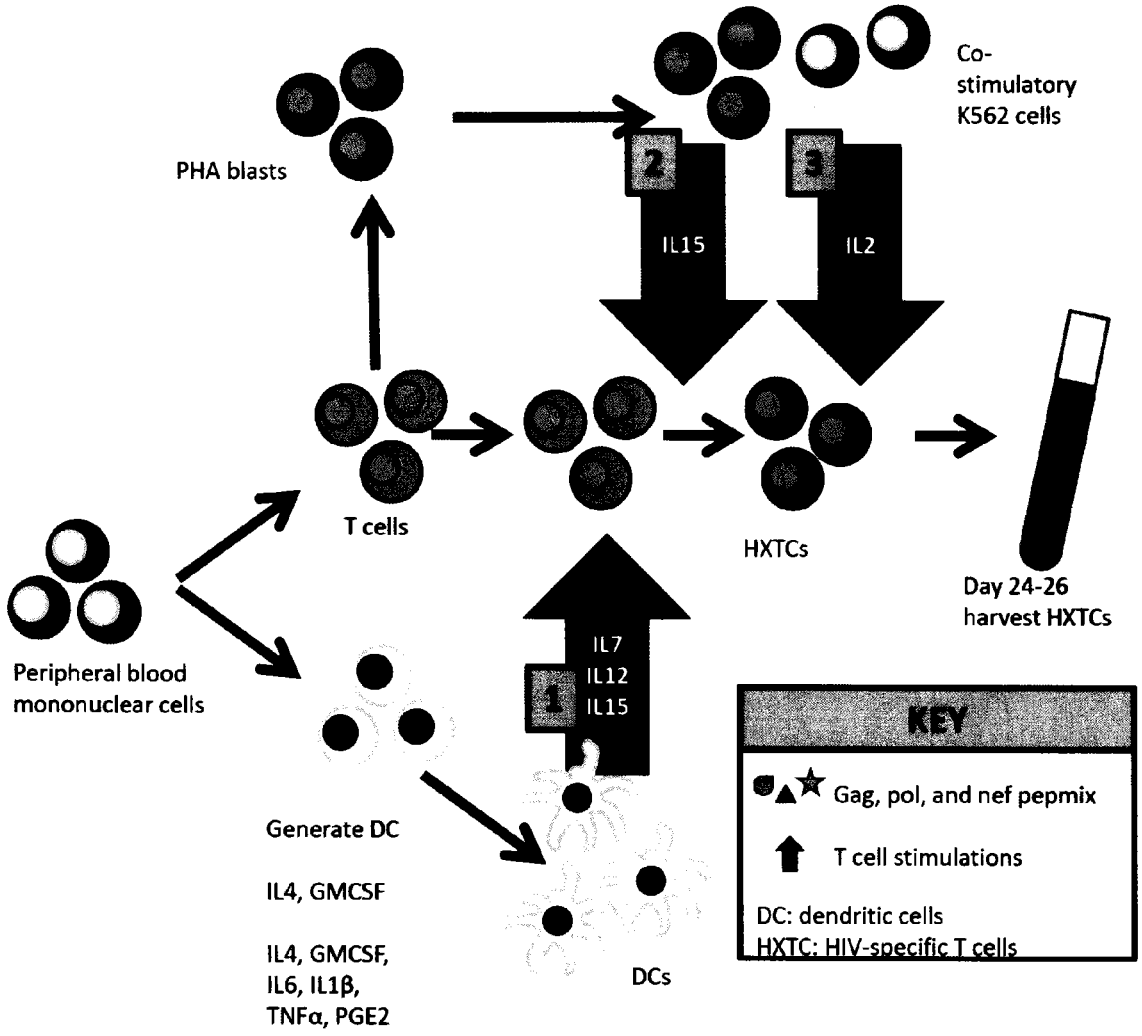
(57) **ABSTRACT**

Compositions for T cell-based immunotherapy of HIV, HIV-associated malignancies, HIV-associated viral infections, or other HIV-related complications. Modified T cells that are resistant to invasion or infection with HIV, such as T-cells modified to decrease or eliminate expression of mannosyl-oligosacharide glucosidase enzyme ("MOGS"). Methods for producing such compositions by expanding HIV-specific T cells from different sources to recognize multiple HIV antigens.

Figure 1


Co-
stimulatory
K562 cells
PHA blasts
2
3
IL15
IL2
T cells
HXTCs
Day 24-26
harvest HXTCs
Peripheral blood
mononuclear cells
IL7
IL12
IL15
1
KEY
Gag, pol, and nef pepmix
T cell stimulations
DC: dendritic cells
HXTC: HIV-specific T cells
Generate DC
IL4, GMCSF
IL4, GMCSF,
IL6, IL1β,
TNFα, PGE2
DCs

GENERATION OF BROADLY-SPECIFIC, VIRUS-IMMUNE CELLS TARGETING MULTIPLE HIV ANTIGENS FOR PREVENTIVE AND THERAPEUTIC USE

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Application 62/011,393, filed Jun. 12, 2014, the contents of which are incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention is focused on the fields of immunotherapy and HIV/AIDS therapeutics. The invention directs a cell product that simultaneously targets multiple HIV antigens while remaining immune to the virus, the method used to generate this product, and its use in preventing and treating active and latent HIV infection, as well as HIV-associated malignancies. More specifically, the invention discloses a method for generation and ex vivo expansion of HIV-antigen specific T-cells that are treated or modified to decrease or eliminate expression of mannosyl-oligosacharide glucosidase enzyme (MOGS) thus rendering them resistant to infection by HIV.

[0004] 2. Description of Related Art

[0005] Antiretroviral therapy (ART) prolongs the life of HIV-infected individuals by preventing the progression to severe immunodeficiency but ART cannot cure infection, and lifelong therapy is necessary to provide continuous viral suppression. Populations that are at high risk for treatment non-adherence are vulnerable to drug resistance and further transmission of HIV, preventing the eradication of the virus on a global scale. Furthermore, the long-term use of ART can lead to side effects in the renal, hepatic, and cardiovascular systems [1]. Another reason why HIV continues to be a pandemic is the lack of an effective vaccine. The most successful vaccine trial to date only produced a marginally statistically significant efficacy of 31% for HIV prevention [2].

[0006] T-cells have been used to treat virus-associated cancers and viral reactivations post-transplant [3-7]. Although T-cells specific for HIV antigens have been produced, $CD4^+$ T-cells are susceptible to infection by HIV which enters the cell through a CD4-dependent mechanism.

[0007] The mannosyl-oligosacharide glucosidase (MOGS) enzyme is deficient in a disease called congenital disorder of glycosylation type IIb (CDG-IIb), where patients exhibit neurologic defects and hypogammaglobulinemia. Along with these gross manifestations however, is an intriguing resistance to viruses with glycan shields: most notably, HIV and influenza [8].

[0008] Drugs that interfere with host endoplasmic reticulum glucosidase activity have been used to reduce the infectivity of secreted virions [9]. Drugs that interfere with N-glycan processing have been proposed as ways to disrupt the morphogenesis of a broad spectrum of enveloped viruses [10]. Such drugs would also affect processing of N-glycans in host cells.

[0009] The effects of reducing or disabling expression of MOGS on the in vivo and ex vivo viability, robustness, and immunological properties of antigen-specific T-cells, and on the resistance of such T-cells to virus invasion and infection have not been previously reported. Thus, the capacity T-cell immunotherapies using such T-cells to treat cancer, viral diseases and other pathologies involving T-cells were not known.

BRIEF SUMMARY OF THE INVENTION

[0010] The present invention describes a cell product derived from any donor source that is HIV-resistant and simultaneously targets multiple HIV antigens and potentially multiple tumor and viral antigens, the method used to generate this product, and its use for preventing and treating HIV infection, HIV-associated malignancy, and HIV-associated infections. The product consists of non-adherent peripheral blood mononuclear cells that have been stimulated with antigen-presenting cells with peptides representing HIV antigens gag, pol, nef, and env and/or viral and tumor antigens associated with HIV-associated disease. These cells were grown in the presence of activating cytokines and feeder cells genetically modified to express co-stimulatory molecules that promote T cell proliferation and differentiation into effector memory cells. This product can be expanded to (1) mediate systemic resistance to HIV by knockdown of MOGS, and (2) improve anti-HIV, antiviral, and/or antitumor capabilities by combination with other therapies through combination with other cells.

[0011] Our strategy to prevent as well as treat HIV infection involves not only the administration of a vaccine but also the administration of cytotoxic T lymphocytes, (i.e., T cell immunotherapeutics), which have proven successful for the treatment of virus-associated cancers and viral reactivations post-transplant [3-7]. Cytotoxic T lymphocytes are immune cells that are responsible for killing virus-infected cells. They recognize non-self proteins (antigens) that are expressed on target cells during infection and kill by producing a variety of inflammatory proteins that form holes on the cell surface, trigger cell death, and stimulate other immune cells to become activated. Furthermore, they have the ability to become memory cells which produce a more rapid and robust response against viral infection. The activation of such memory cells is responsible for the efficacy of vaccines in preventing infections like influenza, for example.

[0012] T cell immunotherapy utilizes these immune cells because they have the ability to proliferate in vivo, and persist long-term as memory cells. This type of therapy consists of redirecting the specificity of T lymphocytes or enriching for pre-existing antigen-specific T lymphocytes ex vivo and expanding these antigen-specific T lymphocytes from patients until a sufficient number of cells are obtained for reinfusion. While the use of T lymphocytes has proven to be effective in the cancer and post-transplant setting, most T cell therapies for HIV so far have only shown safety without the ability to control viral load long-term [11-13]. In a recent study where T cell expression of the CCR5 HIV entry co-receptor has been abrogated, longer T cell persistence is seen. However, effects on viral load after ART treatment interruption do not show significant decreases from their peak levels with the possible exception of one patient where a 2.1 log decrease was seen. However, this patient has been found to be heterozygous for CCR5 delta32; it is unclear how this has impacted the findings [14].

[0013] Two possible reasons for the decreased efficacy seen with this approach so far are: (1) the use of single epitopes or antigens to target HIV, and (2) the sole use of CD8+ cytotoxic T cells. Using T-cells targeting single epitopes inherently

limits the number of targetable HIV infected cells, and increases the risks for viral escape and subsequent resistance to the immunotherapy. Further, using only CD8+ T cells eliminates the T-cell help provided by CD4+ T cells. Single antigens have traditionally been used because of the difficulty of generating polyclonal populations in culture, thought to be the result of immunodominant antigens competing with less immunogenic antigens, while the sole use of CD8+ T cells allows circumvention of the viral tropism towards CD4+ T cells since infused CD4 T cells will theoretically be additional targets for viral infection.

[0014] Using Multiple Antigens.

[0015] Cells administered in HIV clinical trials thus far have largely been single epitope specific CD3+CD8+ preselected T lymphocyte clones expanded in the presence of mitogens [11, 12]. This is in contrast to the administration of polyclonal virus specific T lymphocytes derived from unselected, peripheral blood mononuclear cells expanded in the presence of whole antigen and growth cytokines that have been successful at targeting EBV [3, 4], CMV, and adenovirus [5-7] in the cancer and post-transplant settings. Hence, we propose that developing an HIV-specific immune cell product containing T lymphocytes with broader recognition would not only increase the ability of the T lymphocytes to target infected cells but also provide antigenic stimulation to enhance the in vivo persistence of these cells. Furthermore, because the majority of these cells generated with our method have a memory phenotype, they can also be infused prior to HIV infection to provide a vaccine-like protection against infection.

[0016] Conferring Immunity to HIV.

[0017] Susceptibility of CD4 T cells to HIV has been addressed by conferring resistance using genetic modification of the T cells. One key mechanism we employed to increase systemic resistance against HIV involves the disruption of the mannosyl-oligosacharide glucosidase enzyme (MOGS) expression.

[0018] A representative MOGS amino acid sequence is encoded by SEQ ID NO: 1 and described by NM_006302.2; GI:149999605 and SEQ ID NO: 2 describes the corresponding MOGS amino acid sequence. Information about these MOGS sequences is incorporated by reference to these accession numbers and database entries (last accessed Jun. 9, 2015). A MOGS amino acid sequence is also described by NCBI Reference Sequence: NP_006293.2; GI: 149999606 (mannosyl-oligosaccharide glucosidase isoform 1 [*Homo sapiens*]; 837 aa protein; Chromosome: 2.Map: 2p13.1). Information about this MOGS sequence is incorporated by reference to these accession numbers and database entries (last accessed Jun. 9, 2015).

[0019] MOGS is a carbohydrate enzyme involved in the processing of N-linked oligosaccharides which is a key process during the coating of the viral particle with its glycan shield. If this process is disrupted, the resulting viral particles have defective glycan shields, and therefore cannot enter CD4 T cells. Moreover, these viral particles are effectively less virulent. The enzyme is deficient in a disease called congenital disorder of glycosylation type IIb (CDG-IIb), where patients exhibit neurologic defects and hypogammaglobulinemia. Along with these gross manifestations however, is an intriguing resistance to viruses with glycan shields: most notably, HIV and influenza [8].

[0020] HIV-specific T cells deficient in MOGS were designed. MOGS may be knocked down in T cells using a combination of any of the following technologies: RNAi, CRISPR, TALENS, zinc finger nucleases, expression of intrabodies, and co-administration of drugs that targets MOGS (castanospermine, N-butyldeoxynojirimycin, or deoxynojirimycin). Knockout or knockdown strategies used for example to remove CCR5 as described by Cannon et al [15] are also incorporated thereto.

[0021] The genetic modification of T cells has been successfully used in the cancer setting, more specifically in the chimeric antigen receptor platform, using a variety of methods (transposon-mediated, retroviral-mediated, lentiviral-mediated)—see Duong C P, et al. [16]which is incorporated by reference.

[0022] In other disease settings, genetic modification does not alter the function of antigen-specific T cells [17]. Thus, we foresee that genetic modification of HIV-specific T cells should confer additional HIV-resistance properties without altering their anti-HIV function.

[0023] Although similar approaches using knockdown of the entry receptors CCR5 and CXCR4 also resulted in HIV-immune T cells, knockdown of these proteins limits cellular resistance to virus strains with tropism towards the relevant receptor.

[0024] Disruption of MOGS expression, on the other hand, would provide a more global systemic resistance towards potentially all strains of HIV. To date, while no demonstrable product demonstrated the potential superiority of this approach, the findings in patients with genetic deficiencies in MOGS were especially encouraging. In these patient cells, MOGS knockdown resulted in fewer viruses released when compared to healthy controls (3.6 to 89 times higher in controls), and infectivity of virus was decreased 50 to 80% [8]. MOGS knockdown will thus theoretically not only confer systemic resistance to the virus, but has the advantage of making newly synthesized virions less virulent.

[0025] Our invention consists of functional, broadly-specific virus-resistant T lymphocytes cell products from both HIV positive and negative individuals generated in a single culture to target gag, pol, nef, and envelope antigens of HIV and/or tumor antigens expressed on HIV-associated tumors, the method we developed to generate this product, and their clinical use.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] FIG. **1**. Schematic of method for generating HIV-specific T cells. The method of expanding HIV-specific T cells is presented, using overlapping peptides spanning the HIV proteins Gag, Pol, Nef, and Env presented by dendritic cells and autologous PHA blasts or CD3/28 blasts. T cells are grown in the presence of indicated cytokines and feeder cells at each stimulation. Peptides are also presented by dendritic cells and autologous PHA blasts or CD3/28 blasts. T cells are grown in the presence of indicated cytokines and feeder cells at each stimulation. MOGS expression or activity in the HIV-antigen specific T-cells recovered by this method has been or is knocked down, attenuated, or knocked out by genetic modification of the T-cells, T-cell precursors, or HIV-antigen specific T-cells or by contacting these cells with one or more drugs or agents that inhibit or block MOGS expression or activity.

DETAILED DESCRIPTION OF THE INVENTION

[0027] "Accessory cell" or "Feeder cell" is a cell, such as a K562 cell, that provides costimulation for recognition of pep-

tide antigens by T-cells or that otherwise assists a T-cell recognize, become primed or expand in the presence of a peptide antigen.

[0028] An "antigen" includes molecules, such as polypeptides, peptides, or glyco- or lipo-peptides that are recognized by the immune system, such as by the cellular or humoral arms of the human immune system. The term "antigen" includes antigenic determinants, such as peptides with lengths of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or more amino acid residues that bind to MHC molecules, form parts of MHC Class I or II complexes, or that are recognized when complexed with such molecules. Examples of antigens include peptides or peptide fragments encoded by HIV gag, pol, nef, and env genes and viral and tumor antigens associated with HIV-associated disease.

[0029] An "antigen presenting cell (APC)" refers to a class of cells capable of presenting one or more antigens in the form of peptide-MHC complex recognizable by specific effector cells of the immune system, and thereby inducing an effective cellular immune response against the antigen or antigens being presented. Examples of professional APCs are dendritic cells and macrophages, though any cell expressing MHC Class I or II molecules can potentially present a peptide antigen.

[0030] A "control" is a reference sample or subject used for purposes of comparison with a test sample or test subject. Positive controls measure an expected response and negative controls provide reference points for samples where no response is expected.

[0031] The term "cytokine" has its normal meaning in the art. Examples of cytokines used in the invention include IL-2, IL-7 and IL-15.

[0032] The term "dendritic cell" or "DC describes a diverse population of morphologically similar cell types found in a variety of lymphoid and non-lymphoid tissues[18]. One embodiment of the invention involves dendritic cells and dendritic cell precursors derived from the blood of an HIV-negative or HIV-positive donor.

[0033] The term "effector cell" describes a cell that can bind to or otherwise recognize an antigen and mediate an immune response. Antigen-specific T-cells are effector cells.

[0034] The term "isolated" means separated from components in which a material is ordinarily associated with, for example, an isolated cord blood mononuclear cell can be separated from red blood cells, plasma, and other components of blood.

[0035] The term "MOGS" refers to the enzyme mannosyl-oligosacharide glucosidase, preferably, human variants of this enzyme. A representative sequence for MOGS is given by SEQ ID NO: 1. MOGS analogs or homologs, such as allelic variants or mammalian homologs to human MOGS, may have 70%, 75%, 80%, 85%, 87.5%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and up to 100% sequence identity or sequence similarity with SEQ ID NO: 1. BLASTP may be used to identify an amino acid sequence having at least 70%, 75%, 80%, 85%, 87.5%, 90%, 92.5%, 95%, 97.5%, 98%, 99% sequence similarity to a reference amino acid sequence, such as that of SEQ ID NO: 1, using a similarity matrix such as BLOSUM45, BLOSUM62 or BLOSUM80. Unless otherwise indicated a similarity score will be based on use of BLOSUM62. When BLASTP is used, the percent similarity is based on the BLASTP positives score and the percent sequence identity is based on the BLASTP identities score. BLASTP "Identities" shows the number and fraction of total residues in the high scoring sequence pairs which are identical; and BLASTP "Positives" shows the number and fraction of residues for which the alignment scores have positive values and which are similar to each other. Amino acid sequences having these degrees of identity or similarity or any intermediate degree of identity or similarity to the amino acid sequences disclosed herein are contemplated and encompassed by this disclosure.

[0036] Nucleic acids encoding MOGS are described by reference to the MOGS amino acid sequences described herein and the genetic code. Such nucleic acids may be produced by chemical synthesis, by molecular biological, or by recombinant methods well known in the art. Such polynucleotides may be incorporated into vectors or DNA constructs and used to knock out or modify the expression of MOGS in a cell. Such MOGS sequences may have 70%, 75%, 80%, 85%, 87.5%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and up to 100% sequence identity with the MOGS sequence of SEQ ID NO: 2. Polynucleotide fragments of such sequences useful for modifying or knocking out cellular MOGS expression also contemplated. Such sequences may be designed to attenuate or knock out MOGS expression or to replace all or part of a MOGS sequence in a cell. The degree of identity between two nucleic acid sequences can be determined using the BLASTn program for nucleic acid sequences, which is available through the National Center for Biotechnology Information (http://_www.ncbi.nlm.nih.gov/blast/Blast.cgi?PAGE=Nucleotides) (last accessed Jun. 9, 2015). The percent identity of two nucleotide sequences may be made using the BLASTn preset "search for short and near exact matches" using a word size of 7 with the filter off, an expect value of 1,000 and match/mismatch of 2/-3, gap costs existence 5, extension 2; or standard nucleotide BLAST using a word size of 11, filter setting "on" (dust) and expect value of 10.

[0037] A "naive" T-cell or other immune effector cell is one that has not been exposed to an antigen or to an antigen-presenting cell presenting a peptide antigen capable of activating that cell.

[0038] A "peptide library" or "overlapping peptide library" within the meaning of the application is a complex mixture of peptides which in the aggregate covers the partial or complete sequence of a protein antigen, especially those of opportunistic viruses. Successive peptides within the mixture overlap each other, for example, a peptide library may be constituted of peptides 15 amino acids in length which overlapping adjacent peptides in the library by 11 amino acid residues and which span the entire length of a protein antigen. Peptide libraries are commercially available and may be custom-made for particular antigens. Methods for contacting, pulsing or loading antigen-presenting cells are well known and incorporated by reference to Ngo, et al.[19].

[0039] The term "precursor cell" refers to a cell which can differentiate or otherwise be transformed into a particular kind of cell. For example, a "T-cell precursor cell" can differentiate or mature into a T-cell and a "dendritic precursor cell" can differentiate or mature into a dendritic cell.

[0040] A "subject" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to humans, simians, equines, bovines, porcines, canines, felines, murines, farm animals, livestock, sport animals, or pets. Subjects include those in need of antigen-specific T-cells resistant to invasion by HIV, such as those

infected by HIV or having AIDS or AIDS-associated opportunistic infections or malignancies.

EMBODIMENTS

[0041] Nonlimiting embodiments of the invention include the following.

[0042] 1. A method for producing HIV-antigen-specific T cell(s) resistant to infection by HIV comprising:

[0043] (a) separating T-cells or T-cell precursors (e.g., CD3+cells or cells that do not adhere to plastic) and dendritic cells or dendritic cell precursors (e.g., CD11C+ cells, CD14+ cells, or cells that do adhere to plastic) in a hematopoietic cell sample,

[0044] (b) producing blasts by contacting a portion of a hematopoietic cell sample or a portion of said separated T-cells or T-cell precursors with PHA or another mitogen, or by CD3/CD28 stimulation, and, optionally, treating the blasts with radiation or another agent to inhibit their outgrowth;

[0045] (c) contacting the dendritic cells or dendritic precursor cells separated in (a) with cytokine(s) or other agent(s) that generate and mature dendritic cells and with at least one HIV peptide antigen to produce HIV-antigen-presenting dendritic cells that present at least one HIV-peptide antigen, and, optionally, treating said HIV-antigen-presenting dendritic cells with radiation or another agent sufficient to inhibit their outgrowth;

[0046] (d) contacting the T-cells or T-cell precursors from (a) with the dendritic antigen-presenting cells produced in (c) in the presence of IL-2, IL-6, IL-7, IL-12, IL-15, and/or IL-21, preferably in the presence of IL-7, IL-12 and/or IL-15 to produce HIV-antigen-specific T-cells that recognize the at least one HIV-peptide antigen;

[0047] (e) contacting HIV-antigen-specific T-cells produced by (d) with the blasts of (b) in the presence of the at least one HIV-peptide antigen, optionally, in the presence of K562 cells, which may express costimulatory molecules, or other accessory or feeder cells and in the presence of IL-2, IL-6, IL-7, IL-12, IL-15, and/or IL-21, and preferably in the presence of IL-2 and/or IL-15;

[0048] (f) optionally, repeating (e) one or more times to restimulate and/or expand the HIV-antigen specific T-cells; and

[0049] (g) recovering HIV-antigen-specific T-cells that recognize the at least one HIV-peptide antigen;

[0050] wherein the expression of mannosyl-oligosacharide glucosidase ("MOGS") in said T-cells, T-cell precursors, or HIV-antigen specific T-cells has been knocked down compared to MOGS expression in otherwise identical cells which has not been knocked down.

[0051] 2. The method of embodiment 1, wherein the hematopoietic cell sample is a cord blood sample or other sample containing naïve immune cells.

[0052] 3. The method of embodiment 1, wherein the hematopoietic cell sample is obtained from a peripheral blood sample from a donor who is HIV-negative.

[0053] 4. The method of embodiment 1, wherein the hematopoietic cell sample is obtained from a peripheral blood sample from a donor who is HIV-positive, who has AIDS, or who has an HIV-associated infection or malignancy.

[0054] 5. The method of embodiment 1, wherein in (b) the blasts are produced using PHA, conconavalin A, pokeweed mitogen, or another mitogen.

[0055] 6. The method of embodiment 1, wherein in (b) the blasts are CD3/CD28 blasts produced by stimulating CD3/CD28.

[0056] 7. The method of embodiment 1, wherein in (b) the blasts are irradiated or chemically treated to prevent their outgrowth.

[0057] 8. The method of embodiment 1, wherein in (c) the separated dendritic cells or dendritic cell precursors are cultured in a dendritic cell medium containing IL-4 and GM-CSF, and then subsequently matured in a dendritic cell medium containing a mixture of IL-4, GM-CSF, IL-1B, IL-4, IL-6, PGE2, and/or TNF-α.

[0058] 9. The method of embodiment 1, wherein in (c) the dendritic cells are contacted with HIV Gag, Pol, Nef and/or Env peptides or HIV Gag, Pol, Nef and/or Env peptide libraries. For example, the dendritic cells or their precursors may be contacted with overlapping peptides spanning the HIV proteins encoded by gag, pol, and nef as sources of antigen presented by dendritic cells in the first stimulation.

[0059] 10. The method of embodiment 1, wherein in (c) the dendritic cells are further contacted with HIV Gag, Pol, Nef and Env peptides or HIV Gag, Pol, Nef and Env peptide libraries.

[0060] 11. The method of embodiment 1, wherein in (d) the T-cells or T-cell precursors from (a) are contacted with the dendritic antigen-presenting cells produced in (c) in the presence of IL-7, IL-12 and IL-15 to produce HIV-antigen-specific T-cells that recognize the at least one HIV-peptide antigen.

[0061] 12. The method of embodiment 1, wherein in (e) the HIV-antigen-specific T-cells from (d) are maintained in a medium containing IL-2.

[0062] 13. The method of embodiment 1, wherein in (e) the HIV-antigen-specific T-cells from (d) are maintained in a medium containing IL-15.

[0063] 14. The method of embodiment 1, wherein in (e) the HIV-antigen-specific T-cells from (d) are contacted with blasts that have been pulsed with HIV Gag, Pol, Nef and/or Env peptides or HIV Gag, Pol, Nef and/or Env peptide libraries.

[0064] 15. The method of embodiment 1, wherein in (e) the HIV-antigen-specific T-cells from (d) are contacted and restimulated with blasts that have been pulsed with HIV Gag, Pol, Nef and/or Env peptides or HIV Gag, Pol, Nef and/or Env peptide libraries at least three times every 5-8 days.

[0065] 16. The method of embodiment 1, wherein the hematopoietic cell sample has been obtained from an HIV-positive subject and steps (d) and/or (e) are performed in a medium containing amprenavir or another drug or agent that inhibits HIV replication.

[0066] 17. The method of embodiment 1, wherein MOGS expression has been knocked down by contacting, maintaining or culturing the T-cells, precursor T-cells or HIV-antigen specific T-cells in a medium containing a drug that inhibits or inactivates MOGS. Examples of such drugs include castanospermine, N-butyldeoxynojirimycin, and deoxynojirimycin.

[0067] 18. The method of embodiment 1, wherein MOGS expression has been knocked down by genetically modifying the T-cell, T-cell precursor, or HIV-antigen specific T-cell to attenuate or knock out MOGS expression; or by modifying the T-cell, T-cell precursor, or HIV-antigen specific T-cell using RNAi or by expression of intrabodies to attenuate or knock out MOGS expression.

[0068] 19. A composition comprising HIV-antigen specific T-cells which recognize two, three, four or more different HIV antigens. This composition may be a cell product, derived from a healthy HIV-seronegative donor, or from an HIV-positive subject or patient with AIDS, expanded ex vivo to allow specific recognition of the HIV antigens encoded by the gag, pol, nef, and env genes, or by any combination of the four. This composition may conveniently be made according to the methods described herein, such as the method of embodiment 1.

[0069] The composition may comprise T-cells or T-cell precursors that recognize antigens other than, or in addition to, HIV antigens, such as antigens from viruses or pathogens associated with HIV infection, such opportunistic pathogens, tumor antigens including HIV-associated tumors, neoplasms or malignancies, or other antigens that can be recognized by T-cells.

[0070] Examples of tumor antigens include cancer testis antigens (survivin, MAGEA4, SSX2, PRAME, NYESO1), pluripotency factors (Oct4, Sox2, Nanog) and tumor protein p53 and MYCN tumor-associated antigen.

[0071] Examples of viral antigens include cytomegalovirus ("CMV") antigens pp65, IE1, UL40, UL103, UL151, UL153, UL28, UL32, UL36, UL55, UL40, UL48, UL82, UL94, UL99, us24, us32; herpes simplex antigens ("HSV") glycoprotein G; Epstein Barr Virus antigens BARF1, BMLF1, BMRF1, BZLF1, EBNALP, EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, gp350/340, LMP1, and LMP2; Human Herpes Virus 8 ("HHV8", which is associated with Kaposi's sarcoma) antigens LNA-1, LANA-1, viral cyclin D, vFLIP, RTA; Human Papilloma Virus 16 ("HPV16") antigens E6, E7, and L1 and Human Papilloma Virus 18 ("HPV16") antigens E6 and E7.

[0072] The cells in the composition may be rendered resistant to HIV infection by knockdown of MOGS. MOGS knockdown may be brought about by recognition of relevant mRNA, such as mRNA encoding MOGS or enzymes necessary for MOG activity, by a complementary RNA molecule, and mediated by RNA interference. For example, molecules encoding interfering RNA (RNAi) may be introduced into a T-cell or T-cell precursor by a suitable vector, such as a lentiviral or retroviral vector.

[0073] Knockdown or disruption of functional expression of MOGS may be brought about by guide DNA recognizing the MOGS gene, packaged with a clustered regularly interspaced short palindromic repeat cas9 or a modified cas9 gene.

[0074] may be accomplished by introducing into a T-cell or T-cell precursor TALENS, CRISPR or zinc-finger nuclease products that disrupt a gene encoding MOGS or a gene necessary for its activity, for example, by transformation or transfection with a lentivirus or retrovirus vector encoding these products.

[0075] Knockdown or disruption of functional expression of MOGS may be brought about by guide DNA recognizing the MOGS gene, packaged with a clustered regularly interspaced short palindromic repeat cas9 or a modified cas9 gene fused with a transcriptional repressor such as KRAB.

[0076] Knockdown or disruption of functional expression of MOGS may be brought about by guide DNA recognizing the MOGS gene, packaged with a clustered regularly interspaced short palindromic repeat dcas9 or a modified dcas9 gene.

[0077] Knockdown or disruption of functional expression of MOGS may also brought about by recognition genomic DNA by engineered transcription activator-like effectors recognizing the MOGS gene

[0078] Knockdown or disruption of functional expression of MOGS can be brought about by introduction of transgenes coding for MOGS-specific intrabodies, for example, by introduction into a T-cell or T-cell precursor a lentivirus or retrovirus vector encoding an intrabody that disrupts MOGS expression or activity.

[0079] Alternatively, a T-cell, T-cell precursor, or antigen-specific T-cell may be co-cultured with a drug that inhibits, blocks or attenuates MOGS expression or activity, such as the drugs castanospermine, N-butyldeoxynojirimycin, or deoxynojirimycin.

[0080] 20. A method for inhibiting HIV invasion and replication in a subject or for treating a subject infected by HIV comprising administering the composition according to embodiment 19, optionally in combination with a drug or agent that attenuates or knocks out MOGS expression, to a subject in need thereof. This method may be used to prevent or treat HIV infections or HIV-associated conditions. A subject may be selected from those who are HIV-negative, but at risk for acquiring an HIV infection, an HIV-positive subject, a patient with AIDS or an HIV-associated malignancy, HIV-associated infection, and a complication of HIV.

[0081] HIV-antigen specific T-cells, such as those produced by the method according to embodiment 1 may be infused into a subject, for example, by intravenous infusion. A single or multiple infusions may be made. Prior to infusion, a subject or patient may be lymphodepleted, for example, by the administration of a drug such as cyclophosphamide, fludarabine, alemtusumab, by other lymphodepleting drugs, or by radiation. Immunomodulatory drugs, such as proteasome inhibitors, monoclonal antibodies, cytokines, anti-inflammatory drugs, or epigenetic-modifying drugs, may be administered to a subject or patient before, during or after an infusion of antigen-specific T-cells. Examples of epigenetic modifying drugs include the classes of histone deacetylase inhibitors and histone demethylase inhibitors.

[0082] Other cellular products may be coadministered with the antigen-specific T-cells according to the invention, such as adipose-derived, bone marrow derived, or dental pulp derived mesenchymal stem cells. Drugs that knockdown MOGS expression, such as castanospermine, N-butyldeoxynojirimycin or deoxynojirimycin may be administered before, during or after administration of antigen-specific T-cells according to the invention.

EXAMPLE

Generation of Virus-Resistant HIV-Specific Cytotoxic T Cells

[0083] Donors

[0084] Blood is collected from HIV-negative and HIV-positive human subjects. Umbilical cord blood is also obtained which is often used as a stem cell source for patients eligible for hematopoietic stem cell transplant. Blood is generally collected in 60 to 100 ml heparinized tubes or EDTA-containing tubes.

[0085] Isolation of Mononuclear Cells

[0086] Peripheral blood mononuclear cells ("PBMCs") are isolated from the blood of HIV-negative and HIV-positive subjects by density gradient centrifugation. The buffy coat

containing PBMCs is removed from sedimented red blood cells and other plasma components and used to produce HIV-antigen specific T-cells. The isolated PBMCs may be preserved for later use by suspension in a cryopreservation medium such as a medium containing fetal bovine serum and dimethylsulfoxide (DMSO) by procedures known in the art.

[0087] Generation of Antigen Presenting Cells

[0088] PBMC were plated on 6 well plates and incubated for 2 hours in dendritic cell media (CellGenix DC media; CellGenix) supplemented with 2 mmol/L GlutaMax (Invitrogen). Nonadherent cells were harvested and cryopreserved. Adherent cells were cultured in dendritic cell media in the presence of interleukin (IL)-4 (1,000 U/mL) and granulocyte macrophage colony-stimulating factor (GM-CSF; 800 U/mL; both R&D). On day 5, immature dendritic cells were matured in dendritic cell media with a cytokine cocktail consisting of IL-4 (1,000 U/mL), GM-CSF (800 U/mL), IL-6 (100 ng/mL), TNF-α (10 ng/mL), IL-1β (10 ng/mL; all R&D), and PGE2 (1 μg/mL; Sigma-Aldrich), and were harvested after 24-48 hours of maturation for use as APC. To generate PHA-blasts, PBMC were stimulated with the mitogen PHA-P (5 μg/mL; Sigma-Aldrich) in presence of IL-2 to promote blast formation (PHA-blasts). PHA-blasts were cultured in RPMI-1640 supplemented with 10% human serum (Valley Medical), 2 mmol/L GlutaMax, and IL-2 (100 U/mL; R&D). To prevent possible viral outgrowth when cells were grown from HIV+ individuals, PHA blasts were cultured in presence of 0.5 ng/mL of amprenavir.

[0089] Generation of HIV-Specific Cytotoxic T Cells (HXTC)

[0090] Matured dendritic cells were harvested and used as APC and simultaneously peptide-pulsed with gag, pol, nef and/or env peptide libraries (PepMix; JPT Peptide Technologies). Dendritic cells were used at a stimulator-to-effector ratio of 1:10. T cells were cultured in RPMI-1640 supplemented with 40% Clicks media (Irvine Scientific), 10% human AB serum, and 2 mmol/L GlutaMax. For initial stimulation, a cytokine mix containing IL-7 (10 ng/mL), IL-12 (10 ng/mL), IL-15 (5 ng/mL) (all R&D) was added. T cells were restimulated with peptide-pulsed autologous irradiated (30 Gy) PHA-blasts at a ratio of 1:4 on day 10 to 12 and cultures were maintained in IL-15 (5 ng/mL)-supplemented media or IL-2 (50 U/mL)-supplemented media and restimulated every 7 days as described previously for 3 stimulation cycles. HXTCs derived from HIV+patients were also expanded in presence of 0.5 ng/mL of amprenavir.

[0091] Generation of HIV-Specific and Tumor/Virus-Specific Cytotoxic T Cells (HXTC-T and HXTC-V)

[0092] Similar to the method above, matured dendritic cells were harvested and used as APC and simultaneously peptide-pulsed with gag, pol, nef and/or env and any combination of the following tumor antigens (survivin, MAGEA4, SSX2, PRAME, Oct4, Sox2, Nanog, p53, MYCN, and NYESO1 peptide libraries) or viral antigens (pp65, IE1, IE1, UL40, UL103, UL151, UL153, UL28, UL32, UL36, UL55, UL40, UL48, UL82, UL94, UL99, us24, us32, us32, HSV-1 glycoprotein G, BARF1, BMLF1, BMRF1, BZLF1, EBNALP, EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, gp350/340, LMP1, LMP2, LNA-1, LANA-1, viral cyclin D, vFLIP, RTA, E6, E7, and L1 peptide libraries) (PepMix; JPT Peptide Technologies). Dendritic cells are used at a stimulator-to-effector ratio of 1:10. T cells were cultured in RPMI-1640 supplemented with 40% Clicks media (Irvine Scientific), 10% human AB serum, and 2 mmol/L GlutaMax. For initial stimulation, a cytokine mix containing IL-7 (10 ng/mL), IL-12 (10 ng/mL), IL-15 (5 ng/mL) (all R&D) was added. T cells are restimulated with peptide-pulsed autologous irradiated (30 Gy) PHA-blasts at a ratio of 1:4 on day 10 to 12 and cultures are maintained in IL-15 (5 ng/mL)-supplemented media or IL-2(50 U/mL)-supplemented media and restimulated every 7 days as described previously for 3 stimulation cycles. HXTCs derived from HIV+ patients are also expanded in presence of 0.5 ng/mL of amprenavir.

[0093] Generation of Virus-Resistant HIV-Specific Cytotoxic T Cells (HXTC-R, HXTC-TR, and HXTC-VR)

[0094] T cells expanded according to the methods (HXTC, HXTC-T, and HXTC-V) above are subjected to disruption of MOGS expression, using any or a combination of the following procedures: RNAi, CRISPR, TALENS, expression of intrabodies, and co-administration of drugs that targets MOGS (castanospermine, N-butyldeoxynojirimycin, or deoxynojirimycin).

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Ala Glu Leu Gly Pro Leu Ala Ala Ser Leu Glu Ala Ala Glu Ser Leu
625                 630                 635                 640

Asp Glu Leu His Trp Ala Pro Glu Leu Gly Val Phe Ala Asp Phe Gly
                645                 650                 655

Asn His Thr Lys Ala Val Gln Leu Lys Pro Arg Pro Pro Gln Gly Leu
            660                 665                 670

Val Arg Val Val Gly Arg Pro Gln Pro Gln Leu Gln Tyr Val Asp Ala
        675                 680                 685

Leu Gly Tyr Val Ser Leu Phe Pro Leu Leu Leu Arg Leu Leu Asp Pro
    690                 695                 700

Thr Ser Ser Arg Leu Gly Pro Leu Leu Asp Ile Leu Ala Asp Ser Arg
705                 710                 715                 720

His Leu Trp Ser Pro Phe Gly Leu Arg Ser Leu Ala Ala Ser Ser Ser
                725                 730                 735

Phe Tyr Gly Gln Arg Asn Ser Glu His Asp Pro Pro Tyr Trp Arg Gly
            740                 745                 750

Ala Val Trp Leu Asn Val Asn Tyr Leu Ala Leu Gly Ala Leu His His
        755                 760                 765

Tyr Gly His Leu Glu Gly Pro His Gln Ala Arg Ala Ala Lys Leu His
    770                 775                 780

Gly Glu Leu Arg Ala Asn Val Val Gly Asn Val Trp Arg Gln Tyr Gln
785                 790                 795                 800

Ala Thr Gly Phe Leu Trp Glu Gln Tyr Ser Asp Arg Asp Gly Arg Gly
                805                 810                 815

Met Gly Cys Arg Pro Phe His Gly Trp Thr Ser Leu Val Leu Leu Ala
            820                 825                 830

Met Ala Glu Asp Tyr
        835
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1. A method for producing HIV-antigen-specific T cells resistant to infection by HIV comprising:

(a) separating T-cells or T-cell precursors from dendritic cells or dendritic cell precursors in a hematopoietic cell sample,

(b) producing blasts by contacting a portion of a hematopoietic cell sample, or a portion of said separated T-cells or T-cell precursors, with PHA or another mitogen, or by CD3/CD28 stimulation, and, optionally, treating the blasts with radiation or another agent to inhibit their outgrowth;

(c) contacting the dendritic cells or dendritic precursor cells separated in (a) with cytokine(s) or other agent(s) that generate and mature dendritic cells and with at least one HIV peptide antigen to produce HIV-antigen-presenting dendritic cells that present at least one HIV-peptide antigen, and, optionally, treating said HIV-antigen-presenting dendritic cells with radiation or another agent sufficient to inhibit their outgrowth;

(d) contacting the T-cells or T-cell precursors from (a) with the dendritic antigen-presenting cells produced in (c) in the presence of IL-7, IL-12 and/or IL-15 to produce HIV-antigen-specific T-cells that recognize the at least one HIV-peptide antigen;

(e) contacting HIV-antigen-specific T-cells produced by (d) with the blasts of (b) in the presence of the at least one HIV-peptide antigen, optionally, in the presence of K562 cells or other accessory cells in the presence of IL-2 and/or IL-15;

(f) optionally, repeating (e) one or more times to restimulate and/or expand the HIV-antigen specific T-cells; and

(g) recovering HIV-antigen-specific T-cells that recognize the at least one HIV-peptide antigen.

2. The method of claim **1**, wherein the hematopoietic cell sample is a cord blood sample or other sample containing naïve immune cells.

3. The method of claim **1**, wherein the hematopoietic cell sample is obtained from a peripheral blood sample from a donor who is HIV-negative.

4. The method of claim **1**, wherein the hematopoietic cell sample is obtained from a peripheral blood sample from a donor who is HIV-positive.

5. The method of claim **1**, wherein in (b) the blasts are produced using PHA, conconavalin A, pokeweed mitogen, or another mitogen.

6. The method of claim **1**, wherein in (b) the blasts are CD3/CD28 blasts produced by stimulating CD3/CD28.

7. The method of claim **1**, wherein in (b) the blasts are irradiated or chemically treated to prevent their outgrowth.

8. The method of claim **1**, wherein in (c) the separated dendritic cells or dendritic cell precursors are cultured in a dendritic cell medium containing IL-4 and GM-CSF, and then subsequently matured in a dendritic cell medium containing a mixture of IL-4, GM-CSF, IL-1B, IL-4, IL-6, PGE2, and/or TNF-α.

9. The method of claim **1**, wherein in (c) the dendritic cells are further contacted with HIV Gag, Pol, Nef and/or Env peptides or HIV Gag, Pol, Nef and/or Env peptide libraries.

10. The method of claim **1**, wherein in (c) the dendritic cells are further contacted with HIV Gag, Pol, Nef and Env peptides or HIV Gag, Pol, Nef and Env peptide libraries.

11. The method of claim **1**, wherein in (d) the T-cells or T-cell precursors from (a) are contacted with the dendritic antigen-presenting cells produced in (c) in the presence of IL-7, IL-12 and IL-15 to produce HIV-antigen-specific T-cells that recognize the at least one HIV-peptide antigen.

12. The method of claim **1**, wherein in (e) the HIV-antigen-specific T-cells from (d) are maintained in a medium containing IL-2.

13. The method of claim **1**, wherein in (e) the HIV-antigen-specific T-cells from (d) are maintained in a medium containing IL-15.

14. The method of claim **1**, wherein in (e) the HIV-antigen-specific T-cells from (d) are contacted with blasts that have been pulsed with HIV Gag, Pol, Nef and/or Env peptides or HIV Gag, Pol, Nef and/or Env peptide libraries.

15. The method of claim **1**, wherein in (e) the HIV-antigen-specific T-cells from (d) are contacted and restimulated with blasts that have been pulsed with HIV Gag, Pol, Nef and/or Env peptides or HIV Gag, Pol, Nef and/or Env peptide libraries at least three times every 5-8 days.

16. The method of claim **1**, wherein the hematopoietic cell sample has been obtained from an HIV-positive subject and steps (d) and/or (e) are performed in a medium containing amprenavir or another drug or agent that inhibits HIV replication.

17. The method of claim **1**, wherein MOGS expression has been knocked down by contacting, maintaining or culturing the T-cells, precursor T-cells or HIV-antigen specific T-cells in a medium containing at least one drug that inhibits or inactivates MOGS.

18. The method of claim **1**, wherein MOGS expression has been knocked down by genetically modifying the T-cell, T-cell precursor, or HIV-antigen specific T-cell to attenuate or knock out MOGS expression; or by modifying the T-cell, T-cell precursor, or HIV-antigen specific T-cell using RNAi or by expression of intrabodies to attenuate or knock out MOGS expression.

19. A composition comprising HIV-antigen specific T-cells produced by the method according to claim **1** which recognize two, three, four or more different HIV antigens.

20. A method for inhibiting HIV invasion and replication in a subject or for treating a subject infected by HIV comprising administering to a subject in need thereof the composition according to claim **19**, optionally in combination with a drug or agent that attenuates or knocks out MOGS expression.

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