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(54) **Title:**
HUMAN ENDOGENOUS RETROVIRUS POLYPEPTIDE COMPOSITIONS AND METHODS OF USE THEREOF

(57) **Abstract:**
HUMAN ENDOGENOUS RETROVIRUS POLYPEPTIDE COMPOSITIONS AND METHODS OF USE THEREOF ABSTRACT The present invention provides isolated HERV polypeptides; and compositions, including immunogenic compositions, comprising a HERV polypeptide. The present invention provides immunogenic compositions comprising a nucleic acid comprising a nucleotide sequence encoding a HERV polypeptide. The immunogenic compositions are useful for stimulating a T cell immune response to a lentiviral peptide. The present invention further provides methods of stimulating an immune response in an individual to a retrovirus- or lentivirus-infected cell. The present invention further provides methods of treating cancers in which HERV polypeptides are expressed. Also provided are methods of treating disorders, involving decreasing an immune response to a HERV polypeptide. FIG. IA

HUMAN ENDOGENOUS RETROVIRUS POLYPEPTIDE COMPOSITIONS AND METHODS OF USE THEREOF

ABSTRACT

The present invention provides isolated HERV polypeptides; and compositions, including immunogenic compositions, comprising a HERV polypeptide. The present invention provides immunogenic compositions comprising a nucleic acid comprising a nucleotide sequence encoding a HERV polypeptide. The immunogenic compositions are useful for stimulating a T cell immune response to a lentiviral peptide. The present invention further provides methods of stimulating an immune response in an individual to a retrovirus- or lentivirus-infected cell. The present invention further provides methods of treating cancers in which HERV polypeptides are expressed. Also provided are methods of treating disorders, involving decreasing an immune response to a HERV polypeptide.

FIG. 1A

HUMAN ENDOGENOUS RETROVIRUS POLYPEPTIDE COMPOSITIONS AND METHODS OF USE THEREOF

CROSS-REFERENCE

This application claims the benefit of U.S. Provisional Patent Application No. 60/832,465, filed July 21, 2006, which application is incorporated herein by reference in its entirety.

BACKGROUND

Human endogenous retrovirus sequences make up 8.29% of the draft human genome. Their prevalence has resulted from the accumulation of past retroviral infectious agents that have entered the germline and established a truce with the host cell. Genes co-opted by the host from endogenous retroviruses are found to be active participants in some cellular processes including viral defense by Fv1 and Fv4 in the mouse, and cellular fusion in human placental development mediated through syncitin. Although-HERV transcripts have been detected in both normal and cancerous tissues, including T cells, their role in normal cell function and carcinogenesis is unclear. While the cellular conditions that promote HERV transcription are not well understood, the APOBECs have been shown to play a role in the control of endogenous retroviruses.

Literature

Griffiths (2001) *Genome Biology* 2:1017.1-1017.5; Müller and De Boer (2006) *PLoS Pathogens* 2:0149; Nelson et al. (2003) *J. Clin. Pathol: Mol. Pathol.* 56:11-18; Contreras-Galindo et al. (2007) *AIDS Res. Human Retrovir.* 23:116-122; U.S. Patent Publication No. 2005/0118573; Rakoff-Nahoum et al. (2006) *AIDS Res. Human Retrovir.* 22:52-56; Schiavetti et al. (2002) *Cancer Res.* 62:5510-5516; Büscher et al. (2005) *Cancer Res.* 65:4172; Clerici et al. (1999) *J. Neuroimmunol.* 99:173.

SUMMARY OF THE INVENTION

The present invention provides isolated HERV polypeptides; and compositions, including immunogenic compositions, comprising a HERV polypeptide. The present invention provides immunogenic compositions comprising a nucleic acid comprising a nucleotide sequence encoding a HERV polypeptide. The immunogenic compositions are useful for stimulating a T cell immune response to a lentiviral peptide. The present invention further provides methods of stimulating an immune response in an individual to a retrovirus- or

lentivirus-infected cell. The present invention further provides methods of treating cancers in which HERV polypeptides are expressed. Also provided are methods of treating disorders, involving decreasing an immune response to a HERV polypeptide.

Brief Description Of The Drawings

Figures 1A and 1B depict expression of HERV-K transcripts in HIV positive and negative individuals' plasma.

Figure 2 depicts HERV/HIV amino acid alignments of HIV HXB-2 and various HERV insertions showing segments of the Gag and Reverse Transcriptase proteins.

Figure 3 depicts ELISPOT T cell responses to HERV and HIV antigens in HIV positive and negative individuals.

Figure 4 depicts an inverse correlation between anti-HERV T cell responses and HIV-1 plasma viral load.

Figure 5 depicts the results of a ^{51}Cr release assay to measure cytotoxicity of HERV-L IQ10-specific $CD8^+$ T cells.

Figure 6 depicts an amino acid sequence of HERV-K reverse transcriptase.

Figure 7A depicts an amino acid sequence of a HERV-L reverse transcriptase.

Figure 7B depicts a nucleotide sequence encoding a HERV-L reverse transcriptase.

Figure 8A depicts an amino acid sequence of a HERV-H envelope.

Figure 8B depicts a nucleotide sequence encoding a HERV-L envelope.

Definitions

A "biological sample" encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents; washed; or enrichment for certain cell populations, such as $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, glial cells, macrophages, tumor cells, peripheral blood mononuclear cells (PBMC), and the like. The term "biological sample" encompasses a clinical sample, and also includes cells in culture, cell supernatants, tissue samples, organs, bone marrow, blood, plasma, serum, cerebrospinal fluid, and the like.

The term "retrovirus" is well known in the art, and includes single-stranded, positive sense, enveloped RNA viruses that include, e.g., the genus Gammaretrovirus (e.g., murine

mammary tumor virus); the genus Epsilonretrovirus; the genus Alpharetrovirus (e.g., avian leukosis virus); the genus Betaretrovirus; the genus Deltaretrovirus (e.g., bovine leukemia virus; human T-lymphotrophic virus (HTLV)); the genus Lentivirus; and the genus Spumavirus. The term "lentivirus," as used herein, refers to a genus of viruses of the Retroviridae family, and includes human immunodeficiency virus-1 (HIV-1); human immunodeficiency virus-2 (HIV-2); simian immunodeficiency virus. (SIV); and feline immunodeficiency virus (FIV).

"Gene delivery vehicle" refers to a construct which is capable of delivering, and, within some embodiments expressing, one or more gene(s) or nucleotide sequence(s) of interest in a host cell. Representative examples of such vehicles include viral vectors, nucleic acid expression vectors, naked DNA, and certain eukaryotic cells (e.g., producer cells).

"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control elements operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control elements need not be contiguous with the coding sequence, so long as they function to direct-the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

As used herein the term "isolated" is meant to describe a polynucleotide, a polypeptide, or a cell that is in an environment different from that in which the polynucleotide, the polypeptide, or the cell naturally occurs. An isolated genetically modified host cell may be present in a mixed population of genetically modified host cells. An isolated polypeptide will in some embodiments be synthetic. "Synthetic polypeptides" are assembled from amino acids, and are chemically synthesized *in vitro*, e.g., cell-free chemical synthesis, using procedures known to those skilled in the art.

By "purified" is meant a compound of interest (e.g., a polypeptide) has been separated from components that accompany it in nature. "Purified" can also be used to refer to a compound of interest separated from components that can accompany it during manufacture (e.g., in chemical synthesis). In some embodiments, a compound is substantially pure when it is at least 50% to 60%, by weight, free from organic molecules with which it is naturally associated or with which it is associated during manufacture. In some embodiments, the preparation is at least 75%, at least 90%, at least 95%, or at least 99%, by weight, of the compound of interest. A substantially pure compound can be obtained, for example, by extraction from a natural source (e.g., bacteria), by chemically synthesizing a compound, or by

a combination of purification and chemical modification. A substantially pure compound can also be obtained by, for example, enriching a sample having a compound that binds an antibody of interest. Purity can be measured by any appropriate method, e.g., chromatography, mass spectroscopy, high performance liquid chromatography analysis, etc.

The term "heterologous," as used herein in the context of a HERV polypeptide, where a HERV polypeptide fusion protein comprises a HERV polypeptide and a heterologous polypeptide, refers to a polypeptide that is other than a HERV polypeptide, e.g., a polypeptide that is not normally associated with a HERV polypeptide. For example, a heterologous polypeptide bears no significant amino acid sequence identity to the HERV antigenic polypeptide, e.g., the heterologous polypeptide has less than about 50%, less than about 40%, less than about 30%, or less than about 20% amino acid sequence identity to the HERV antigenic polypeptide.

An "antigen" is defined herein to include any substance that may be specifically bound by an antibody molecule. An "immunogen" is an antigen that is capable of initiating lymphocyte activation resulting in an antigen-specific immune response.

By "epitope" is meant a site on an antigen to which specific B cells and/or T cells respond. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site." B cell epitope sites on proteins, polysaccharides, or other biopolymers may be composed of moieties from different parts of the macromolecule that have been brought together by folding. Epitopes of this kind are referred to as conformational or discontinuous epitopes, since the site is composed of segments of the polymer that are discontinuous in the linear sequence but are continuous in the folded conformation(s). Epitopes that are composed of single segments of biopolymers or other molecules are termed continuous or linear epitopes. T cell epitopes are generally linear peptides. Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen.

The terms "cancer," "neoplasm," and "tumor" are used interchangeably herein to refer to cells which exhibit relatively autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. Cells of interest for treatment in the present application include precancerous, malignant, pre-metastatic, metastatic, and non-metastatic cells, as well as carcinoma *in situ*.

"Cancerous phenotype" generally refers to any of a variety of biological phenomena that are characteristic of a cancerous cell, which phenomena can vary with the type of cancer. The cancerous phenotype is generally identified by abnormalities in, for example, cell growth

or proliferation (*e.g.*, uncontrolled growth or proliferation), regulation of the cell cycle, cell mobility, cell-cell interaction, or metastasis, etc.

The terms "subject," "individual," "host," and "patient" are used interchangeably herein to refer to a mammal, including, but not limited to, murines (rats, mice), felines, non-human primates (e.g., simians), humans, canines, ungulates, etc.

The terms "treatment," "treating," "treat," and the like are used herein to generally refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete stabilization or cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease or symptom from occurring in a subject which may be predisposed to the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease symptom, i.e., arresting its development; or (c) relieving the disease symptom, i.e., causing regression of the disease or symptom.

Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by

reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

It must be noted that as used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a human endogenous retrovirus polypeptide" includes a plurality of such polypeptides and reference to "the immunogenic composition" includes reference to one or more immunogenic compositions and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

The present invention provides isolated HERV polypeptides; and compositions, including immunogenic compositions, comprising a HERV polypeptide. The present invention provides immunogenic compositions comprising a nucleic acid comprising a nucleotide sequence encoding a HERV polypeptide. The immunogenic compositions are useful for stimulating a T cell immune response to a lentiviral peptide. The present invention further provides methods of stimulating an immune response in an individual to a retrovirus- or lentivirus-infected cell. The present invention further provides methods of treating cancers in which HERV polypeptides are expressed by cancerous cells. Also provided are methods of treating disorders, involving decreasing an immune response to a HERV polypeptide.

In some embodiments, a subject immunogenic composition induces a T cell immune response specific for a lentivirus-infected cell, e.g., a human immunodeficiency virus (HIV)-infected cell. Epitopes displayed by a HERV polypeptide stimulate or enhance a T cell immune response to the epitopes. Where the HERV epitopes are also present on the surface of a lentivirus-infected cell, a T cell response to the lentivirus-infected cell also occurs. A "T cell immune response" includes one or more of: 1) an increase in the number and/or activity of $CD4^+$ T cells specific for the HERV epitope; 2) an increase in the number and/or activity (e.g.,

cytotoxicity) of $CD8^+$ T cells specific for the HERV epitope; and 3) secretion of cytokines that induce or are indicative of a Th2-type immune response. Cytokines that induce or are indicative of a Th2 immune response include, but are not limited to, interferon-gamma (IFN-γ), IL-2, and tumor necrosis factor-alpha (TNF-α). T cell immune responses that are stimulated with a subject immunogenic composition include a mucosal T cell immune response and a systemic T cell immune response.

A subject immunogenic composition may be formulated in any of a variety of ways, including a formulation suitable for intravenous administration, subcutaneous administration, or other parenteral route of administration; a formulation suitable for administration to a mucosal tissue; and the like. The present invention provides pharmaceutical formulations comprising a subject immunogenic composition.

The present invention further provides HERV polypeptide compositions that are suitable for use in monitoring a patient's response to treatment for a lentivirus infection (e.g., an HIV infection). Thus, the present invention further provides methods for monitoring a patient's response to treatment for a lentivirus infection (e.g., an HIV infection).

ISOLATED HERV POLYPEPTIDES

The present invention provides isolated HERV polypeptides, and compositions comprising the HERV polypeptides. Isolated HERV polypeptides find use in, e.g., generating immunogenic compositions (e.g., for enhancing an immune response in an individual to a HERV polypeptide); generating immunomodulatory compositions (e.g., for reducing an immune response in an individual to a HERV polypeptide; monitoring patient response to therapy, e.g., therapy for a lentivirus infection; staging a disease; detecting a disease; and for generating $CD8^+$ T cells for adoptive transfer methods.

HERV polypeptides

HERV polypeptides include polypeptides encoded by any HERV class or group, e.g., of HERV-W, HERV-H, HERV-K, HERV-L, and HERV-S, and any subgroup thereof. HERV classes, groups, and subgroups are known in the art. See, e.g., Griffiths (2001) *Genome Biology* 2:1017.1-1017.5.

In some embodiments, a subject isolated HERV polypeptide comprises a polypeptide comprising from about 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 50, from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, or from 350 to 400, or more, contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about

98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence of a HERV-encoded polypeptide. HERV-encoded polypeptides include a polypeptide encoded by the Gag-Pro-Pol region, and a polypeptide encoded by the env region of a HERV.

In some embodiments, a subject isolated HERV polypeptide comprises a stretch of from about 9, 10, 11, 12, 13-15, 15-17, 17-20, or from 20 to 25, or more contiguous amino acids having at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to a stretch of the same length in an HIV-encoded protein.

A subject isolated HERV polypeptide can be from 9 amino acids in length up to the length of a naturally-occurring HERV polypeptide, e.g., a HERV polypeptide can be 9 amino acids (aa), 10 aa, 11 aa, 12-15 aa, 15-20 aa, 20-25 aa, 25-30 aa, 30-40 aa, 40-50 aa, 50-100 aa, or longer than 100 amino acids, e.g., 100 aa to 150 aa, 150 aa to 200 aa.

Exemplary, non-limiting examples of HERV-encoded polypeptides are found in GenBank Accession Nos. AAD51797 (HERV-K Gag-Pro-Pol protein); AAD51798 (HERV-K env protein); CAA13576; AJ233632; AF108843; etc.

In some embodiments, a subject isolated HERV polypeptide comprises a polypeptide comprising from about 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 50, from 50 to 75, from 75 to 80, or from 80 to 87 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:26:

IIIDLKDCFFTIPLAEQDCEKFAFTIPAINNKEPATRFQWKVLPQGMLNSPTICQT FVGRALQPVREKFSDCYIIHCIDDILCAAET (SEQ ID NO:26).

In some embodiments, a subject isolated HERV polypeptide comprises a polypeptide comprising from about 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 30, from 30 to 35, from 35 to 40, or from 40 to 43 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 27: AAIDLANAFFSIPVHKAHKKQFAFTICVYCPASGVYQQSSFVS (SEQ ID NO:27).

In some embodiments, a subject isolated HERV polypeptide comprises a polypeptide comprising from about 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 30, from 30 to 35, from 35 to 40, from 40 to 45, from 45 to 50, from 50 to 55, or from 55 to 58 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:28: FAFRWQGQQYSFTVLSQGYINSPALCHNLIQRELDHFLLLQDIILVHYIDDIMLIGSS (SEQ ID NO:28).

In some embodiments, a subject isolated HERV polypeptide comprises a polypeptide comprising from about 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 30, from 30 to 35, from 35 to 40, from 40 to 45, from 45 to 50, from 50 to 55, from 55 to 60, from 60 to 65, or from 65 to 71 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:29: KLRLPPGYFGLLLHLSQQAMKGVTVLAGVIDLDYQDEISLLLHNRGKEEYAWNTGDP LGCLLVLPCPVIKV (SEQ ID NO:29).

In some embodiments, a subject isolated HERV polypeptide comprises a polypeptide comprising from about 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 30, or from 30 to 35 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:30:

YTHDRAQAVPEGTSKLHEEVAQMPMVSTPATLSLP (SEQ ID NO:30).

In some embodiments, a subject isolated HERV polypeptide comprises a polypeptide comprising from about 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 30, from 30 to 35, from 35 to 50, or from 50 to 100, or more, contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NOs:31, 32, and 34, e.g., as depicted in Figures 6, 7A, and 8A, respectively.

In some embodiments, a subject isolated HERV polypeptide comprises one or more of he following amino acid sequences:

SQGYINSPAL (SEQ ID NO:1);

ILVHYIDDI (SEQ ID NO:2);

LQDIILVHY (SEQ ID NO:3);

PMVSTPATL (SEQ ID NO:4);

AAIDLANAF (SEQ ID NO:5);

IPVHKAHKKQ (SEQ ID NO:6);

SSGLMLMEF (SEQ ID NO:7);

KIRLPPGYF (SEQ ID NO:8);

DSIEGQLILK (SEQ ID NO:9);

FAFTIPAI (SEQ ID NO:10);

GIPYNSQGQ (SEQ ID NO:11);

FEGLVDTGAD (SEQ ID NO:12);

FLQFKTWWI (SEQ ID NO:13);

VPLTKEQVR (SEQ ID NO:14);

LDLLTAEKGGLCI (SEQ ID NO:15);

TLEPIPPGE (SEQ ID NO:16);

DPLAPLQLL (SEQ ID NO:17);

KLLGDINWI (SEQ ID NO:18);

LPHSTVKTF (SEQ ID NO:19);

GPGYCSKAF (SEQ ID NO:20);

IPTRHLKFY (SEQ ID NO:21);

VPSFGRLSY (SEQ ID NO:22);

PPTVEARYK (SEQ ID NO:23);

PPESQYGYP (SEQ ID NO:24); and

YPQPPTRRL (SEQ ID NO:25).

In certain embodiments, the following peptides are specifically excluded: FLQFKTWWI (SEQ ID NO:13); PPESQYGYP (SEQ ID NO:24); and PTVEARYK (SEQ ID NO:23).

Fusion proteins

In some embodiments, a subject isolated HERV polypeptide is a fusion protein, e.g., a HERV fusion protein comprises a HERV polypeptide covalently linked to a heterologous protein, where the heterologous protein is also referred to as a "fusion partner." In some

embodiments, the fusion partner is attached to the N-terminus of the HERV protein, e.g., NH_2-fusion partner- HERV-COOH. In other embodiments, the fusion partner is attached to the C-terminus of the HERV protein, e.g., NH_2- HERV-fusion partner-COOH. In other embodiments, the fusion partner is internal to the HERV protein, e.g., NH_2-$(HERV)_1$-FP-$(HERV_2$-$COOH)_2$, where FP is a fusion partner, and $HERV_1$ and $HERV_2$ are N-terminal and C-terminal regions, respectively, of HERV.

Suitable fusion partners include, but are not limited to, immunological tags such as epitope tags, including, but not limited to, hemagglutinin, FLAG, myc, and the like; proteins that provide for a detectable signal, including, but not limited to, fluorescent proteins, enzymes (e.g., β-galactosidase, luciferase, horse radish peroxidase, alkaline phosphatase, etc.), and the like; polypeptides that facilitate purification or isolation of the fusion protein, e.g., metal ion binding polypeptides such as 6His tags, glutathione-S-transferase, and the like; polypeptides that provide for subcellular localization; and polypeptides that provide for secretion from a cell. Fusion partners that provide for a detectable signal are also referred to as "reporters." In some embodiments, a fusion partner is an immunomodulatory polypeptide other than a HERV polypeptide, e.g., an antigen, a cytokine, etc.

Multimerized HERV polypeptides

In some embodiments, a subject isolated HERV polypeptide is multimerized, e.g., two or more HERV polypeptides are linked in tandem. Multimers include dimers, trimers, tetramers, pentamers, etc. Monomeric HERV polypeptides are linked to one another directly or via a linker. Thus, in some embodiments, a subject HERV polypeptide has the formula $(X_1$-$(Y)_{0\text{-}40}$-X_2-$(Y)_{0\text{-}40})_n$, where X_1 and X_2 are HERV polypeptides, Y is a linker, and n is an integer from 1 to about 10 (e.g., n = 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10). Where a linker is used, Y is one or more amino acids, or other linking groups. X_1 and X_2 can be the same or different, e.g., can have the same amino acid sequence, or can differ from one another in amino acid sequence. Thus, e.g., a subject HERV polypeptide can have the formula X_1-$(Y)_{0\text{-}40}$-X_2, e.g., where the HERV polypeptide is a dimer. As another example, a subject HERV polypeptide can have the formula X_1-$(Y)_{0\text{-}40}$-X_2-$(Y)_{0\text{-}40}$-X_3, e.g., where the HERV polypeptide is a trimer.

Where Y is a spacer peptide, it is generally of a flexible nature, although other chemical linkages are not excluded. Currently, it is contemplated that the most useful linker sequences will generally be peptides of between about 2 and about 40 amino acids in length, e.g., from about 2 amino acids to about 10 amino acids , from about 10 amino acids to about 20 amino acids, or from about 6 amino acids to about 25 amino acids in length. These linkers are generally produced by using synthetic, linker-encoding oligonucleotides to couple the proteins.

Peptide linkers with a degree of flexibility will generally be used. The linking peptides may have virtually any amino acid sequence, bearing in mind that the preferred linkers will have a sequence that results in a generally flexible peptide. The use of small amino acids, such as glycine and alanine, are of use in creating a flexible peptide. Exemplary peptide linkers include $(Gly)_{2-40}$, $(Ser)_{2-40}$, and $(Ala)_{2-40}$. The creation of such sequences is routine to those of skill in the art. A variety of different linkers are commercially available and are considered suitable for use according to the present invention. However, any flexible linker generally between about 2 amino acids and about 40 amino acids, e.g., from about 6 amino acids to about 10 amino acids in length may be used. Linkers may have virtually any sequence that results in a generally flexible peptide.

Linkages for homo- or hetero-polymers or for coupling to carriers can be provided in a variety of ways. For example, cysteine residues can be added at both the amino- and carboxyl-termini, where the peptides are covalently bonded via controlled oxidation of the cysteine residues. Also useful are a large number of heterobifunctional agents which generate a disulfide link at one functional group end and a peptide link at the other, including N-succidimidyl-3-(2-pyridyldithio) proprionate (SPDP). This reagent creates a disulfide linkage between itself and a cysteine residue in one protein and an amide linkage through the amino on a lysine or other free amino group in the other. A variety of such disulfide/amide forming agents are known. See, for example, Immun. Rev. 62:185 (1982). Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these thioether forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2 bromoacetic acid, 2-iodoacetic acid, 4-(N-maleimido-methyl) cyclohexane-1-carboxylic acid and the like. The carboxyl groups can be activated by combining them with succinimide or 1-hydroxy-2-nitro-4-sulfonic acid, sodium salt. A particularly preferred coupling agent is succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC). Of course, it will be understood that linkage should not substantially interfere with either of the linked groups to function for its intended use, e.g., as an immunogen.

Carriers

In some embodiments, a subject isolated HERV polypeptide is linked to a carrier. The term "linked," as used herein interchangeably with the term "coupled," refers to proximately associated, e.g., the HERV polypeptide and the carrier are in close spatial proximity. In some embodiments, the linkage is a covalent linkage. In other embodiments, the linkage is a non-covalent linkage. In some embodiments, the HERV polypeptide is linked directly to the

carrier. In other embodiments, the HERV polypeptide is linked indirectly, e.g., via a linker molecule.

Examples of suitable carriers include large, slowly metabolized macromolecules such as: proteins; polysaccharides, such as sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids such as polyglutamic acid, polylysine, and the like; amino acid copolymers; inactivated virus particles; inactivated bacterial toxins such as toxoid from diphtheria, tetanus, cholera, leukotoxin molecules; liposomes; inactivated bacteria; dendritic cells; and the like. Carriers are described in further detail below.

Suitable carriers are well known in the art, and include, e.g., thyroglobulin, albumins such as human serum albumin, tetanus toxoid; Diphtheria toxoid; polyamino acids such as poly(D-lysine:D-glutamic acid); VP6 polypeptides of rotaviruses; influenza virus hemagglutinin, influenza virus nucleoprotein; hepatitis B virus core protein, hepatitis B virus surface antigen; purified protein derivative (PPD) of tuberculin from *Mycobacterium tuberculosis*; inactivated *Pseudomonas aeruginosa* exotoxin A (toxin A); Keyhole Limpet Hemocyanin (KLH); filamentous hemagglutinin (FHA) of *Bordetella pertussis*; T helper cell (Th) epitopes of tetanus toxoid (TT) and Bacillus Calmette-Guerin (BCG) cell wall; recombinant 10 kDa, 19 kDa and 30-32 kDa proteins from *M. leprae* or from *M. tuberculosis*, or any combination of these proteins; and the like. See, e.g., U.S. Patent No. 6,447,778 for a discussion of carriers methods of conjugating peptides to carriers.

Pseudomonas aeruginosa exotoxin A (toxin A) has been used effectively as a carrier in conjugate vaccines. *Pseudomonas aeruginosa* exotoxin A may be purified from the supernatant of fermentor-grown cultures of *Pseudomonas aeruginosa* PA 103. Toxin A has been classified as a superantigen based upon results in animals. Toxin A can be completely and irreversibly detoxified by covalent coupling to adipic acid dihydrazide (ADH), a 4 carbon spacer molecule. This step destroys the ADPR-transferase activity of the toxin molecule, hence rendering it nontoxic. The non-reacted hydrazide group can be used to covalently couple a polypeptide to toxin A. Toxin A may also be coupled to a polypeptide using a carbodiimide reagent.

PPD-peptide conjugates are conveniently prepared with glutaraldehyde as coupling agent. See, e.g., Rubinstein et al. (1995) *AIDS* 9:243-51.

The methods by which a subject polypeptide is conjugated with a carrier include disulfide linkages through a C terminal peptide cysteine linkage, coupling with glutaraldehyde solution for two hours, coupling with tyrosine, or coupling with water soluble carbodiimide.

In some embodiments, a subject isolated HERV polypeptide is lipidated. Lipidation increases a cytotoxic T cell (CTL) response to the peptide that is linked to the lipid. The lipid

residue, such as palmitic acid or the like, is attached to the amino terminus of the peptide. The lipid can be attached directly to the peptide, or, indirectly via a linkage, such as a Ser-Ser, Gly, Gly-Gly, Ser linkage or the like. As another example, *E. coli* lipoprotein, such as tripalmitoyl-S-glycerylcysteinyl-seryl-serine (P_3 CSS), can be used to prime specific CTL when covalently attached to the peptide. See, Deres et al., *Nature* 342:561-564 (1989). A HERV polypeptide can be conjugated with uncharged fatty acid residues of different chain lengths and degrees of unsaturation, ranging from acetic to stearic acid as well as to negatively charged succinyl residues via the appropriate carboxylic acid anhydrides. See, e.g., U.S. Patent No. 6,419,931.

A subject isolated HERV polypeptide may be conjugated directly or indirectly, e.g., via a linker molecule, to a carrier. A wide variety of linker molecules are known in the art and can be used in the conjugates. The linkage from the peptide to the carrier may be through a peptide reactive side chain, or the N- or C-terminus of the peptide. A linker may be an organic, inorganic, or semi-organic molecule, and may be a polymer of an organic molecule, an inorganic molecule, or a co-polymer comprising both inorganic and organic molecules.

If present, the linker molecules are generally of sufficient length to permit the HERV polypeptide and a linked carrier to allow some flexible movement between the HERV polypeptide and the carrier. The linker molecules are generally about 6-50 atoms long. The linker molecules may also be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Other linker molecules which can bind to polypeptides may be used in light of this disclosure.

Compositions

The present invention provides compositions comprising a subject isolated HERV polypeptide. Compositions comprising a HERV polypeptide can include one or more of: a salt, e.g., NaCl, MgCl, KCl, $MgSO_4$, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a protease inhibitor; and the like. In some embodiments, as described in more detail below, a subject HERV composition is an immunogenic composition. In other embodiments, as described in more detail below, a subject HERV composition is a pharmaceutical composition, e.g., a composition comprising a HERV polypeptide and a pharmaceutically acceptable excipient.

In some embodiments, a subject composition comprises a single type (or "species") of HERV polypeptide, e.g., in some embodiments, the HERV polypeptides in a subject composition all comprise substantially the same amino acid sequence. In other embodiments, a subject immunogenic composition comprises two or more different HERV polypeptides, e.g., the composition comprises a population of HERV polypeptides, the member of which population can differ in amino acid sequence. A subject composition can comprise from two to about 20 different HERV polypeptides, e.g., a subject composition can comprise two, three, four, five, six, seven, eight, nine, ten, 11-15, or 15-20 different HERV polypeptides, each having an amino acid that differs from the amino acid sequences of the other HERV polypeptides. For example, in some embodiments, a subject composition comprises a first HERV polypeptide having a first amino acid sequence; and at least a second HERV polypeptide having a second amino acid sequence, where the second amino acid sequence differs from the first amino acid sequence. As another example, in some embodiments, a subject composition comprises a first HERV polypeptide having a first amino acid sequence; second HERV polypeptide having a second amino acid sequence, where the second amino acid sequence differs from the first amino acid sequence; and at least a third HERV polypeptide having a third amino acid sequence, where the third amino acid sequence differs from both the first and the second amino acid sequences. In other embodiments, a subject composition comprises a multimerized HERV polypeptide, as described above.

Production of HERV polypeptides

A subject HERV polypeptide can be produced in a number of ways, including, e.g., by chemical synthesis, where the HERV polypeptide is a "synthetic" polypeptide; by isolation and purification from a naturally-occurring source; and by recombinant means, where the HERV polypeptide is a "recombinant" polypeptide. Recombinant means for producing a HERV polypeptide are well known in the art, and involve genetically modifying a host cell with a polynucleotide comprising a nucleotide sequence encoding a HERV polypeptide, culturing the host cell *in vitro* under conditions and for a suitable time such that the HERV polypeptide is produced by the genetically modified cell, and isolating the HERV polypeptide produced by the genetically modified cell.

IMMUNOGENIC COMPOSITIONS COMPRISING A HERV POLYPEPTIDE

The present invention provides immunogenic compositions, comprising a HERV polypeptide, e.g., a polypeptide comprising amino acid sequences derived from or related to a human endogenous retrovirus (HERV) polypeptide. HERV polypeptides suitable for inclusion in a subject immunogenic composition are as described above.

In some embodiments, a subject immunogenic composition comprises a HERV polypeptide that comprises one or more T cell epitopes that, when presented on the surface of a lentivirus-infected cell, induce a T cell immune response specific for a lentivirus-infected cell, e.g., a human immunodeficiency virus (HIV)-infected cell. A "T cell immune response" includes one or more of: 1) an increase in the number and/or activity of $CD4^+$ T cells specific for the HERV epitope; 2) an increase in the number and/or activity of $CD8^+$ T cells specific for the HERV epitope; and 3) secretion of cytokines that induce or are indicative of a Th2-type immune response. Cytokines that induce or are indicative of a Th2 immune response include, but are not limited to, interferon-gamma (IFN-γ), IL-2, and tumor necrosis factor-alpha (TNF-α).

A subject immunogenic composition comprising a subject HERV polypeptide can be formulated in a number of ways, as described in more detail below. In some embodiments, a subject immunogenic composition comprises single species of HERV polypeptide, e.g., the immunogenic composition comprises a population of HERV polypeptides, substantially all of which have the same amino acid sequence. In other embodiments, a subject immunogenic composition comprises two or more different HERV polypeptides, e.g., the immunogenic composition comprises a population of HERV polypeptides, the member of which population can differ in amino acid sequence. A subject immunogenic composition can comprise from two to about 20 different HERV polypeptides, e.g., a subject immunogenic composition can comprise two, three, four, five, six, seven, eight, nine, ten, 11-15, or 15-20 different HERV polypeptides, each having an amino acid that differs from the amino acid sequences of the other HERV polypeptides. For example, in some embodiments, a subject immunogenic composition comprises a first HERV polypeptide having a first amino acid sequence; and at least a second HERV polypeptide having a second amino acid sequence, where the second amino acid sequence differs from the first amino acid sequence. As another example, in some embodiments, a subject immunogenic composition comprises a first HERV polypeptide having a first amino acid sequence; second HERV polypeptide having a second amino acid sequence, where the second amino acid sequence differs from the first amino acid sequence; and at least a third HERV polypeptide having a third amino acid sequence, where the third amino acid sequence differs from both the first and the second amino acid sequences. In other embodiments, a subject immunogenic composition comprises a multimerized HERV polypeptide, as described above.

Adjuvants

The immunogenic compositions to be administered are provided in a pharmaceutically acceptable diluent such as an aqueous solution, e.g., a saline solution, a semi-solid form (e.g., gel), or in powder form. Such diluents can be inert, although a subject HERV composition may also include an adjuvant. Examples of known suitable adjuvants that can be used in humans include, but are not necessarily limited to, alum, aluminum phosphate, aluminum hydroxide, MF59 (4.3% w/v squalene, 0.5% w/v Tween 80, 0.5% w/v Span 85), CpG-containing nucleic acid (where the cytosine is unmethylated), QS21, MPL, 3DMPL, extracts from Aquilla, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, Quil A, interleukins, and the like. For non-human animals (e.g. for veterinary applications; for experimental non-human animals), one can use Freund's, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic antigen.

Further exemplary adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (W090/14837; Chapter 10 in *Vaccine design: the subunit and adjuvant approach,* eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80 (polyoxyethylene sorbitan mono-oleate), and 0.5% Span 85 (sorbitan trioleate) (optionally containing muramyl tri-peptide covalently linked to dipalmitoyl phosphatidylethanolamine (MTP-PE)) formulated into submicron particles using a microfluidizer, (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) RIBI™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components such as monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DETOX™); (2) saponin adjuvants, such as QS21 or STIMULON™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMS may be devoid of additional detergent *e.g.*

WO00/07621; (3) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins *(e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (WO99/44636), *etc.),* interferons *(e.g.* gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), *etc.;* (5) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) *e.g.* GB-2220221, EP-A-0689454, optionally in the substantial absence of alum when used with pneumococcal saccharides *e.g.* WO00/56358; (6) combinations of 3dMPL with, for example, QS21 and/oroil-in-water emulsions *e.g.* EP-A-0835318, EP-A-0735898, EP-A-0761231; (7) oligonucleotides comprising CpG motifs [Krieg *Vaccine* 2000, 19, 618-622; Krieg *Curr opin Mol Ther2001* 3:15-24; Roman *etal., Nat. Med.,* 1997, 3, 849-854; Weiner *et a., PNAS USA,* 1997, 94, 10833-10837; Davis *et al, J. Immunol,* 1998, 160, 870-876; Chu *et a., J. Exp.Med,* 1997, 186, 1623-1631; Lipford *et al, Ear. J. Immunol.,* 1997, 27, 2340-2344; Moldoveami e/ *al., Vaccine,* 1988, 16, 1216-1224, Krieg *etal., Nature,* 1995, 374, 546-549; Klinman *et al., PNAS USA,* 1996, 93, 2879-2883; Ballas *et al, J. Immunol,* 1996, 157, 1840-1845; Cowdery *et al, J. Immunol,* 1996, 156, 4570-4575; Halpern *et al, Cell Immunol,* 1996, 167, 72-78; Yamamoto *et al, Jpn. J. Cancer Res.,* 1988, 79, 866-873; Stacey *et al, J. Immunol.,* 1996, 157,2116-2122; Messina *et al, J. Immunol,* 1991, 147, 1759-1764; Yi *et al, J. Immunol,* 1996, 157,4918-4925; Yi *et al, J. Immunol,* 1996, 157, 5394-5402; Yi *et al, J. Immunol,* 1998, 160, 4755-4761; and Yi *et al, J. Immunol,* 1998, 160, 5898-5906; International patent applications WO96/02555, W098/16247, WO98/18810, WO98/40100, WO98/55495, WO98/37919 and WO98/52581] *i.e.* containing at least one CG dinucleotide, where the cytosine is unmethylated; (8) a polyoxyethylene ether or a polyoxyethylene ester *e.g.* WO99/52549; (9) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (WO01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (WO01/21152); (10) a saponin and an immunostimulatory oligonucleotide *(e.g.* a CpG oligonucleotide) (WO00/62800); (11) an immunostimulant and a particle of metal salt *e.g.* WO00/23105; (12) a saponin and an oil-in-water emulsion *e.g.* WO99/11241; (13) a saponin *(e.g.* QS21) + 3dMPL + IM2 (optionally + a sterol) *e.g.* WO98/57659; (14) other substances that act as immunostimulating agents to enhance the efficacy of the composition. Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-25 acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), *etc.*

The immunogenic compositions may be combined with a conventional pharmaceutically acceptable excipient, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium,

carbonate, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of antigen in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs. The resulting compositions may be in the form of a solution, suspension, tablet, pill, capsule, powder, gel, cream, lotion, ointment, aerosol or the like.

The protein concentration of a subject immunogenic in the pharmaceutical formulations can vary widely, i.e. from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

In some embodiments, a HERV polypeptide is formulated with one or more lipids. For example, liposomes of various sizes can be made. Small liposomes or vesicles formed are unilamellar and have a size in the range of about 20 to 400 nanometers and can be produced by subjecting multi-lamellar vesicles to ultrasound, by extrusion under pressure through membranes having pores of defined size, or by high pressure homogenization. Larger unilamellar liposomes having a size in the range of about 0.1 to 1 μm in diameter can be obtained when the lipid is solubilized in an organic solvent or a detergent and the solubilized agent is removed by evaporation or dialysis, respectively. The fusion of smaller unilamellar liposomes by methods requiring particular lipids or stringent dehydration-hydration conditions can yield unilamellar vessels as large or larger than cells.

Liposomes may comprise one or more cationic lipids, e.g., DDAB, dimethyldioctadecyl ammonium bromide; N-[1-(2,3-Dioloyloxy)propyl]-N,N,N-trimethylammonium methylsulfate; 1,2-diacyl-3-trimethylammonium-propanes, (including but not limited to, dioleoyl (DOTAP), dimyristoyl, dipalmitoyl, disearoyl); 1,2-diacyl-3-dimethylammonium- propanes, (including but not limited to, dioleoyl, dimyristoyl, dipalmitoyl, disearoyl) DOTMA, N-[1-[2,3-bis(oleoyloxy)]propyl]-N,N,N-trimethylammonium chloride; DOGS, dioctadecylamidoglycylspermine; DC-cholesterol, 3β-[N-(N',N'-dimethylaminoethane)carbamoyl]cholesterol; DOSPA, 2,3-dioleoyloxy-N-(2(sperminecarboxamido)- ethyl)-N,N-dimethyl-1-propanaminium trifluoroacetate; 1,2-diacyl-sn-glycero-3-ethylphosphocholines (including but not limited to dioleoyl (DOEPC), dilauroyl, dimyristoyl, dipalmitoyl, distearoyl, palmitoyl-oleoyl); β-alanyl

cholesterol; CTAB, cetyl trimethyl ammonium bromide; diC14-amidine, N-t-butyl-N'-tetradecyl-3- tetradecylaminopropionamidine; 14Dea2, O,O'-ditetradecanolyl-N-(trimethylammonioacetyl) diethanolamine chloride; DOSPER, 1,3-dioleoyloxy-2-(6-carboxy-spermyl)-propylamide; N,N,N',N'-tetramethyl-N,N'-bis(2-hydroxylethyl)-2,3-dioleoyloxy-1,4-butanediammonium iodide; 1-[2-acyloxy)ethyl]2-alkyl (alkenyl)-3-(2-hydroxyethyl)imidazolinium chloride derivatives such as 1-[2-(9(Z)-octadecenoyloxy)ethyl]-2-(8(Z)-heptadecenyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), 1-[2-(hexadecanoyloxy)ethyl]-2-pentadecyl-3-(2-hydroxyethyl)imidazolinium chloride (DPTIM); 1-[2-tetradecanoyloxy)ethyl]-2-tridecyl-3-(2-hydroxyethyl)imidazolium chloride (DMTIM) - as described in Solodin et al. (1995) Biochem. 43:13537-13544; 2,3-dialkyloxypropyl quaternary ammonium compound derivates, containing a hydroxyalkyl moiety on the quaternary amine, such as 1,2-dioleoyl-3-dimethyl- hydroxyethyl ammonium bromide (DORI); 1,2-dioleyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE); 1,2-dioleyloxypropyl-3-dimethyl- hydroxypropyl ammonium bromide (DORIE-HP); 1,2-dioleyloxypropyl-3-dimethyl-hydroxybutyl ammonium bromide (DORIE-HB); 1,2-dioleyloxypropyl-3-dimethyl- hydroxypentyl ammonium bromide (DORIE-HPe); 1,2-dimyristyloxypropyl-3-dimethyl-hydroxylethyl ammonium bromide (DMRIE); 1,2-dipalmityloxypropyl-3- dimethyl-hydroxyethyl ammonium bromide (DPRIE); 1,2-disteryloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DSRIE) - as described, e.g., in Felgner et al. (1994) *J. Biol. Chem.* 269:2550-2561. Many of the above-mentioned lipids are available commercially from, e.g., Avanti Polar Lipids, Inc.; Sigma Chemical Co.; Molecular Probes, Inc.; Northern Lipids, Inc.; Roche Molecular Biochemicals; and Promega Corp.

Liposomes may comprise cationic lipids alone, or in admixture with other lipids, particularly neutral lipids such as: cholesterol; 1,2-diacyl-sn-glycero-3-phosphoethanolamines, (including but not limited to dioleoyl (DOPE), 1,2-diacyl-sn-glycero-3-phosphocholines; natural egg yolk phosphatidyl choline (PC), and the like; synthetic mono- and diacyl phosphocholines (e.g., monoacyl phosphatidyl choline (MOPC)) and phosphoethanolamines. Asymmetric fatty acids, both synthetic and natural, and mixed formulations, for the above diacyl derivatives may also be included.

Other suitable liposome compositions include dimyristoylphosphatidylcholine (DMPC) and cholesterol. Such liposomes are described in, e.g., U.S. Patent No. 5,916,588. Additional

suitable liposomal compositions, and methods of preparing same, are known in the art, and are described in various publications, including, e.g., U.S. Patent Nos. 4,241,046 and 6,355,267.

IMMUNOGENIC COMPOSITIONS COMPRISING HERV POLYNUCLEOTIDES

The present invention provides an immunogenic composition comprising a HERV polynucleotide, e.g., a polynucleotide comprising a nucleotide sequence encoding a HERV polypeptide. When administered to an individual in need thereof, the polynucleotide (the "HERV polynucleotide") comprising a nucleotide sequence encoding a HERV polypeptide is taken up by a cell, e.g., an antigen-presenting cell, the encoded HERV polypeptide is produced in the cell, and the HERV polypeptide is processed into epitope-displaying polypeptide fragments ("epitope fragments") that are then displayed on the surface of the cell in association with an MHC molecule. The encoded HERV polypeptide stimulates or enhances a T cell response to the epitope(s) displayed on the cell surface. Where the HERV epitopes are also present on a lentivirus-infected cell, a T cell response to the lentivirus-infected cell also occurs.

Expression vectors and delivery vehicles

In some embodiments, a HERV polynucleotide is an expression vector. The expression vector will provide a transcriptional and translational initiation region, which may be inducible or constitutive, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region.

Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding heterologous proteins. A selectable marker operative in the expression host may be present. Suitable expression vectors include, but are not limited to, viral vectors (e.g. viral vectors based on vaccinia virus; poliovirus; adenovirus (see, e.g., Li et al., Invest Opthalmol Vis Sci 35:2543 2549, 1994; Borras et al., Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto et al., H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (see, e.g., Ali et al., Hum Gene Ther 9:81 86, 1998, Flannery et al., PNAS 94:6916 6921, 1997; Bennett et al., Invest Opthalmol Vis Sci 38:2857 2863, 1997; Jomary et al., Gene Ther 4:683 690, 1997, Rolling et al., Hum Gene Ther 10:641 648, 1999; Ali et al., Hum Mol Genet 5:591 594, 1996; Srivastava in WO 93/09239, Samulski et al., J. Vir. (1989) 63:3822-3828; Mendelson et al., Virol. (1988) 166:154-165; and Flotte et al., PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, e.g., Miyoshi et al., PNAS 94:10319 23, 1997; Takahashi et al., J Virol 73:7812 7816, 1999); a retroviral vector (e.g., Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma

Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

Numerous suitable expression vectors are known to those of skill in the art, and many are commercially available. The following vectors are provided by way of example; for eukaryotic host cells: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, and pSVLSV40 (Pharmacia). However, any other vector may be used so long as it is compatible with the host cell.

Depending on the host/vector system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (see e.g., Bitter et al. (1987) *Methods in Enzymology*, 153:516-544).

Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. The expression vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The expression vector may also include appropriate sequences for amplifying expression.

A subject recombinant vector will in some embodiments include one or more selectable markers. In addition, the expression vectors will in many embodiments contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture.

Other gene delivery vehicles and methods may be employed, including polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example Curiel (1992) *Hum. Gene Ther.* 3:147-154; ligand linked DNA, for example see Wu (1989) *J. Biol. Chem.* 264:16985-16987; eukaryotic cell delivery vehicles cells; deposition of photopolymerized hydrogel materials; hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; ionizing radiation as described in U.S. Patent No. 5,206,152 and in WO 92/11033; nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol. Cell Biol.* 14:2411-2418, and in Woffendin (1994) *Proc. Natl. Acad. Sci.* 91:1581-1585.

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and U.S. Patent No. 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by

treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm. Liposomes that can act as gene delivery vehicles are described in U.S. Patent No. 5,422,120, PCT Nos. WO 95/13796, WO 94/23697, and WO 91/14445, and EP No. 524 968.

Liposome or lipid nucleic acid delivery vehicles can also be used. Liposome complexes for gene delivery are described in, e.g., U.S. Patent No. 7,001,614. For example, liposomes comprising DOTAP and at least one cholesterol and/or cholesterol-derivative, present in a molar ratio range of 2.0 mM 10 mM provide an effective delivery system, e.g., where the molar ratio of DOTAP to cholesterol is 1:1 3:1. The cationic lipid N-[(2,3-dioleoyloxy)propyl]-L-lysinamide (LADOP) can be used in a composition for delivering a HERV polynucleotide, where LADOP-containing liposomes are described in, e.g., U.S. Patent No. 7,067,697. Liposome formulations comprising amphipathic lipids having a polar headgroup and aliphatic components capable of promoting transfection are suitable for use and are described in, e.g., U.S. Patent No. 6,433,017.

Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin et al.(1994) *Proc. Natl. Acad. Sci. USA* 91:11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. Patent No. 5,206,152 and PCT No. WO 92/11033.

TREATMENT METHODS

The present invention provides various treatment methods, which methods utilize a subject HERV polypeptide or a subject HERV composition. Subject treatment methods include methods of inducing an immune response in an individual to a HERV polypeptide, and methods of enhancing a subject's immune response to a HERV polypeptide, e.g., for the treatment of a retrovirus infection (e.g., a lentivirus infection), for the treatment of cancer, etc; and methods for reducing subject's immune response to a HERV polypeptide, e.g., for the treatment of an autoimmune disorder, for the treatment of schizophrenia, etc.

Methods of inducing or enhancing an immune response to a retrovirus-infected cell

The present invention provides methods for inducing, eliciting, or enhancing a T cell immune response to a retrovirus-infected cell, e.g., an HTLV-infected cell, in an individual in

need thereof. The methods generally involve administering an effective amount of a subject immunogenic composition to the individual.

In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, reduces retroviral load in the individual by at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 50%, at least about 75%, at least about 85%, or at least about 90%, compared to the viral load in the individual before treatment with the immunogenic composition.

In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of T cells specific for a retrovirus epitope present on a retrovirus-infected cell. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of T cells specific for a retrovirus epitope present on a retrovirus-infected cell, compared with the number of T cells specific for a retrovirus epitope in the individual before treatment with the immunogenic composition.

In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of $CD8^+$ T cells specific for a retrovirus epitope present on a retrovirus-infected cell. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of $CD8^+$ T cells specific for a retrovirus epitope present on a retrovirus-infected cell, compared with the number of $CD8^+$ T cells specific for a retrovirus epitope in the individual before treatment with the immunogenic composition.

In some embodiments, e.g., where the immunogenic composition is administered to a naïve individual (i.e., an individual not infected with a retrovirus such as HTLV), an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, reduces the likelihood that the individual, if later infected with a retrovirus such as HTLV, would develop disease symptoms from the retrovirus infection. In some embodiments, e.g., where the immunogenic composition is administered to a naïve

individual (i.e., an individual not infected with a retrovirus), an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, increases the likelihood that the individual, if later infected with a retrovirus such as HIV, would limit and/or clear the retrovirus infection.

Methods of inducing or enhancing an immune response to a lentivirus-infected cell

The present invention provides methods for inducing, eliciting, or enhancing a T cell immune response to a lentivirus-infected cell, e.g., an HIV-infected cell, in an individual in need thereof. The methods generally involve administering an effective amount of a subject immunogenic composition to the individual.

In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, reduces viral load in the individual by at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 50%, at least about 75%, at least about 85%, or at least about 90%, compared to the viral load in the individual before treatment with the immunogenic composition.

In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase in $CD4^+$ T lymphocyte levels and function(s) in the individual. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, compared to the level of $CD4^+$ T lymphocytes in the individual before treatment with the immunogenic composition. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in a number of $CD4^+$ T lymphocytes that is within the normal range, where the normal range for humans is from about 600 to about 1500 $CD4^+$ T lymphocytes per mm^3 blood.

In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of T cells specific for a lentivirus epitope present on a lentivirus-infected cell. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of T cells specific for a lentivirus epitope present on a lentivirus-infected cell, compared with the number of T cells

specific for a lentivirus epitope in the individual before treatment with the immunogenic composition.

In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of $CD8^+$ T cells specific for a lentivirus epitope present on a lentivirus-infected cell. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of $CD8^+$ T cells specific for a lentivirus epitope present on a lentivirus-infected cell, compared with the number of $CD8^+$ T cells specific for a lentivirus epitope in the individual before treatment with the immunogenic composition.

In some embodiments, e.g., where the immunogenic composition is administered to a naïve individual (i.e., an individual not infected with a lentivirus such as HIV), an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, reduces the likelihood that the individual, if later infected with a lentivirus such as HIV, would develop disease symptoms from the lentivirus infection. In some embodiments, e.g., where the immunogenic composition is administered to a naïve individual (i.e., an individual not infected with a lentivirus such as HIV), an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, increases the likelihood that the individual, if later infected with a lentivirus such as HIV, would limit and/or clear the lentivirus infection.

Combination therapies

A subject immunogenic composition can be administered in conjunction with one or more therapeutic agents for the treatment of a lentiviral infection, or for the treatment of a disorder that may accompany a lentiviral infection (e.g., a bacterial infection, a fungal infection, and the like). Therapeutic agents beta-lactam antibiotics, tetracyclines, chloramphenicol, neomycin, gramicidin, bacitracin, sulfonamides, nitrofurazone, nalidixic acid, cortisone, hydrocortisone, betamethasone, dexamethasone, fluocortolone, prednisolone, triamcinolone, indomethacin, sulindac, acyclovir, amantadine, rimantadine, recombinant soluble CD4 (rsCD4), anti-receptor antibodies (e.g., for rhinoviruses), nevirapine, cidofovir (Vistide™), trisodium phosphonoformate (Foscarnet™), famcyclovir, pencyclovir, valacyclovir, nucleic acid/replication inhibitors, interferon, zidovudine (AZT, Retrovir™), didanosine (dideoxyinosine, ddI, Videx™), stavudine (d4T, Zerit™), zalcitabine

(dideoxycytosine, ddC, Hivid™), nevirapine (Viramune™), lamivudine (Epivir™, 3TC), protease inhibitors, saquinavir (Invirase™, Fortovase™), ritonavir (Norvir™), nelfinavir (Viracept™), efavirenz (Sustiva™), abacavir (Ziagen™), amprenavir (Agenerase™) indinavir (Crixivan™), ganciclovir, AzDU, delavirdine (Rescriptor™), kaletra, trizivir, rifampin, clathiromycin, erythropoietin, colony stimulating factors (G-CSF and GM-CSF), non-nucleoside reverse transcriptase inhibitors, nucleoside inhibitors, adriamycin, fluorouracil, methotrexate, asparaginase and combinations thereof.

Methods of treating cancer

The present invention further provides methods of treating cancer in an individual, where the cancer is associated with expression of HERV. Such cancers include, but are not limited to, ovarian cancer, breast cancer, melanoma, prostate cancer, seminoma, teratoma, and testicular cancer. The methods generally involved administering to an individual in need thereof an effective amount of a subject immunogenic composition comprising one or more HERV polypeptides.

A subject method for treating cancer is useful for treating cancer that derived from a tissue comprising cells that normally express one or more HERV polypeptides. Such cancers include ovarian cancer, breast cancer, melanoma, prostate cancer, seminoma, teratoma, and testicular cancer.

In some embodiments, in the context of cancer treatment, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, reduces one or more of tumor size, cancer cell number, and cancer cell metastasis by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about80%, or at least about 90%, up to total eradication of the tumor.

In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of T cells specific for an epitope present on a cancer cell. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of T cells specific for an epitope present on a cancer cell, compared with the number of T cells specific for a cancer cell epitope in the individual before treatment with the immunogenic composition.

In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of $CD8^+$ T cells specific for an epitope present on a cancer cell. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of $CD8^+$ T cells specific for a an epitope present on a cancer cell, compared with the number of $CD8^+$ T cells specific for a cancer cell epitope in the individual before treatment with the immunogenic composition.

In some embodiments, a subject immunogenic composition is administered as an adjuvant therapy to a standard cancer therapy. Standard cancer therapies include surgery (e.g., surgical removal of cancerous tissue), radiation therapy, bone marrow transplantation, chemotherapeutic treatment, biological response modifier treatment, and certain combinations of the foregoing.

Radiation therapy includes, but is not limited to, x-rays or gamma rays that are delivered from either an externally applied source such as a beam, or by implantation of small radioactive sources.

Chemotherapeutic agents are non-peptidic (i.e., non-proteinaceous) compounds that reduce proliferation of cancer cells, and encompass cytotoxic agents and cytostatic agents. Non-limiting examples of chemotherapeutic agents include alkylating agents, nitrosoureas, antimetabolites, antitumor antibiotics, plant (vinca) alkaloids, and steroid hormones.

Agents that act to reduce cellular proliferation are known in the art and widely used. Such agents include alkylating agents, such as nitrogen mustards, nitrosoureas, ethylenimine derivatives, alkyl sulfonates, and triazenes, including, but not limited to, mechlorethamine, cyclophosphamide (Cytoxan™), melphalan (L-sarcolysin), carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin, uracil mustard, chlormethine, ifosfamide, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, dacarbazine, and temozolomide.

Antimetabolite agents include folic acid analogs, pyrimidine analogs, purine analogs, and adenosine deaminase inhibitors, including, but not limited to, cytarabine (CYTOSAR-U), cytosine arabinoside, fluorouracil (5-FU), floxuridine (FudR), 6-thioguanine, 6-mercaptopurine (6-MP), pentostatin, 5-fluorouracil (5-FU), methotrexate, 10-propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, fludarabine phosphate, pentostatine, and gemcitabine.

Suitable natural products and their derivatives, (e.g., vinca alkaloids, antitumor antibiotics, enzymes, lymphokines, and epipodophyllotoxins), include, but are not limited to, Ara-C, paclitaxel (Taxol®), docetaxel (Taxotere®), deoxycoformycin, mitomycin-C, L-asparaginase, azathioprine; brequinar; alkaloids, *e.g.* vincristine, vinblastine, vinorelbine, vindesine, *etc.*; podophyllotoxins, *e.g.* etoposide, teniposide, *etc.*; antibiotics, *e.g.* anthracycline, daunorubicin hydrochloride (daunomycin, rubidomycin, cerubidine), idarubicin, doxorubicin, epirubicin and morpholino derivatives, *etc.*; phenoxizone biscyclopeptides, *e.g.* dactinomycin; basic glycopeptides, *e.g.* bleomycin; anthraquinone glycosides, *e.g.* plicamycin (mithramycin); anthracenediones, *e.g.* mitoxantrone; azirinopyrrolo indoledione, *e.g.* mitomycin; macrocyclic immunosuppressants, *e.g.* cyclosporine, FK-506 (tacrolimus, prograf), rapamycin, *etc.*; and the like.

Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrazole, letrazole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine.

Microtubule affecting agents that have antiproliferative activity are also suitable for use and include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (e.g., NSC 33410), dolstatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (Taxol®), Taxol® derivatives, docetaxel (Taxotere®), thiocolchicine (NSC 361792), trityl cysterin, vinblastine sulfate, vincristine sulfate, natural and synthetic epothilones including but not limited to, eopthilone A, epothilone B, discodermolide; estramustine, nocodazole, and the like.

Hormone modulators and steroids (including synthetic analogs) that are suitable for use include, but are not limited to, adrenocorticosteroids, *e.g.* prednisone, dexamethasone, *etc.*; estrogens and pregestins, *e.g.* hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, estradiol, clomiphene, tamoxifen; *etc.*; and adrenocortical suppressants, *e.g.* aminoglutethimide; 17α-ethinylestradiol; diethylstilbestrol, testosterone, fluoxymesterone, dromostanolone propionate, testolactone, methylprednisolone, methyl-testosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, Flutamide (Drogenil), Toremifene (Fareston), and Zoladex®. Estrogens stimulate proliferation and differentiation; therefore, compounds that bind to the estrogen receptor are used to block this activity. Corticosteroids may inhibit T cell proliferation.

Other chemotherapeutic agents include metal complexes, *e.g.* cisplatin (cis-DDP), carboplatin, *etc.*; ureas, *e.g.* hydroxyurea; and hydrazines, *e.g.* N-methylhydrazine; epidophyllotoxin; a topoisomerase inhibitor; procarbazine; mitoxantrone; leucovorin; tegafur;

etc.. Other anti-proliferative agents of interest include immunosuppressants, *e.g.* mycophenolic acid, thalidomide, desoxyspergualin, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685); Iressa® (ZD 1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy)quinazoline); etc.

"Taxanes" include paclitaxel, as well as any active taxane derivative or pro-drug. "Paclitaxel" (which should be understood herein to include analogues, formulations, and derivatives such as, for example, docetaxel, TAXOL™, TAXOTERE™ (a formulation of docetaxel), 10-desacetyl analogs of paclitaxel and 3'N-desbenzoyl-3'N-t-butoxycarbonyl analogs of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (see also WO 94/07882, WO 94/07881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076; U.S. Pat. Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; and EP 590,267), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Mo. (T7402 from *Taxus brevifolia*; or T-1912 from *Taxus yannanensis*).

Paclitaxel should be understood to refer to not only the common chemically available form of paclitaxel, but analogs and derivatives (e.g., Taxotere™ docetaxel, as noted above) and paclitaxel conjugates (e.g., paclitaxel-PEG, paclitaxel-dextran, or paclitaxel-xylose).

Also included within the term "taxane" are a variety of known derivatives, including both hydrophilic derivatives, and hydrophobic derivatives. Taxane derivatives include, but not limited to, galactose and mannose derivatives described in International Patent Application No. WO 99/18113; piperazino and other derivatives described in WO 99/14209; taxane derivatives described in WO 99/09021, WO 98/22451, and U.S. Patent No. 5,869,680; 6-thio derivatives described in WO 98/28288; sulfenamide derivatives described in U.S. Patent No. 5,821,263; and taxol derivative described in U.S. Patent No. 5,415,869. It further includes prodrugs of paclitaxel including, but not limited to, those described in WO 98/58927; WO 98/13059; and U.S. Patent No. 5,824,701.

Biological response modifiers suitable for use in connection with the methods of the invention include, but are not limited to, (1) inhibitors of tyrosine kinase (RTK) activity; (2) inhibitors of serine/threonine kinase activity; (3) tumor-associated antigen antagonists, such as antibodies that bind specifically to a tumor antigen; (4) apoptosis receptor agonists; (5) interleukin-2; (6) IFN-α; (7) IFN-γ (8) colony-stimulating factors; and (9) inhibitors of angiogenesis.

Methods for treating autoimmune disorders

The present invention provides methods of treating an autoimmune disorder in an individual, the methods generally involving administering to an individual in need thereof an amount of a subject HERV polypeptide effective to reduce a subject's immune response to a HERV polypeptide, thereby treating the autoimmune disease. Autoimmune disorders that can be treated with a subject method include, but are not limited to, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and Type 1 diabetes.

In some embodiments, an effective amount of a subject HERV polypeptide is an amount that is effective to reduce a subject's immune response to a HERV polypeptide by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or more than 50%, compared to the level of the subject's immune response to the HERV polypeptide in absence of treatment with a subject HERV polypeptide.

In some embodiments, a subject method is effective in reducing autoreactivity, where "reducing autoreactivity" includes one or more of reducing the number of autoreactive cells; reducing the activity of an autoreactive cell; and reducing the level of autoreactive antibody. Autoreactivity depends on the interactions of a number of white blood cells, including but not limited to, T lymphocytes, B cells, natural killer (NK) cells and dendritic cells. T lymphocytes include $CD4^+$ T lymphocytes and $CD8^+$ lymphocytes. B cells can function both as antigen presenting cells and producers of autoantibodies that can target tissues. In some embodiments, the subject method can alter the activities or numbers of these cells involved in various autoimmune reactivities. In some embodiments, a subject method is effective to reduce the number and/or activity of an autoreactive cell in an individual by at least about 5%, at least about 10%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, or more, when compared to the number and/or level of autoreactive cells in the individual not treated with the HERV polypeptide.

In some embodiments, a subject method is effective to reduce the number and/or activity of an autoreactive T lymphocyte. Thus, in some embodiments, an effective amount of a HERV polypeptide is an amount that is effective to reduce the number and/or activity of autoreactive T lymphocytes in an individual by at least about 5%, at least about 10%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, or more, when compared to the

number and/or level of autoreactive T lymphocytes in the individual not treated with the HERV polypeptide.

In some embodiments, a subject method is effective to reduce the number and/or activity of an autoreactive B cell. Thus, in some embodiments, an effective amount of a HERV polypeptide is an amount that is effective to reduce the number and/or activity of autoreactive B cells in an individual by at least about 5%, at least about 10%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, or more, when compared to the number and/or level of autoreactive B cells in the individual not treated with the HERV polypeptide.

Activities of an autoreactive T lymphocyte include, but are not limited to, cytolytic activity toward a "self" cell; secretion of cytokine(s); secretion of chemokine(s); responsiveness to chemokine(s); and trafficking. In some embodiments, an effective amount of a HERV polypeptide is an amount that is effective to reduce one or more activities of an autoreactive T lymphocyte in an individual.

Whether a HERV polypeptide is effective to reduce the number and/or activity of an autoreactive T lymphocyte in an individual is readily determined using known assays. For example, where the autoreactive T lymphocytes are specific for an autoantigen, the number and activity level of autoantigen-specific T lymphocytes is determined using, e.g., a mixed lymphocyte reaction in which irradiated cells comprising a detectable label in the cytoplasm and displaying the autoantigen are mixed with lymphocytes from the individual. Release of detectable label from the cytoplasm of the autoantigen-displaying cells indicates the presence in the individual of autoreactive lymphocytes. Methods of detecting autoreactive T lymphocytes associated with Type 1 diabetes are known in the art; and any such methods can be used. See, e.g., U.S. Patent No. 6,022,697 for a discussion of a method of detecting autoreactive T lymphocytes associated with Type 1 diabetes.

In some embodiments, an effective amount of a HERV polypeptide is an amount that is effective to reduce the severity of one or more symptoms of an autoimmune disease. For example, in some embodiments, an effective amount of a HERV polypeptide is an amount that is effective to reduce the severity of one or more symptoms of an autoimmune disease by at least about 5%, at least about 10%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, or more, when compared to the severity of the symptom in an individual not treated with the HERV polypeptide.

Symptoms associated with autoimmune disorders are known in the art. See, e.g., "Textbook of the Autoimmune Diseases" R.G. Lahita, Ed. (2000) Lippincott Williams & Wilkins, 1st ed. The following are non-limiting examples.

Multiple sclerosis is characterized by various symptoms and signs of central nervous system (CNS) dysfunction, with remissions and recurring exacerbations. The most common presenting symptoms are paresthesias in one or more extremities, in the trunk, or on one side of the face; weakness or clumsiness of a leg or hand; or visual disturbances, *e.g.* partial blindness and pain in one eye (retrobulbar optic neuritis), dimness of vision, or scotomas. Other common early symptoms are ocular palsy resulting in double vision (diplopia), transient weakness of one or more extremities, slight stiffness or unusual fatigability of a limb, minor gait disturbances, difficulty with bladder control, vertigo, and mild emotional disturbances.

Diabetes Mellitus is syndrome characterized by hyperglycemia resulting from absolute or relative impairment in insulin secretion and/or insulin action. Although it may occur at any age, type I DM most commonly develops in childhood or adolescence and is the predominant type of DM diagnosed before age 30. This type of diabetes accounts for 10 to 15% of all cases of DM and is characterized clinically by hyperglycemia.

Combination therapies

In some embodiments, a subject treatment method will involve administering to an individual in need thereof an effective amount of a HERV polypeptide; and at least one additional agent that is effective for the treatment of an autoimmune disorder. In some embodiments, the at least one additional agent is other than a HERV polypeptide.

Those skilled in the art are aware of agents (other than a HERV polypeptide) that are suitable for treating autoimmune disorders. For example, agents that are suitable for treating Type 1 diabetes include insulin, including naturally occurring insulin, insulin analogs, and the like.

Insulin that is suitable for use herein includes, but is not limited to, regular insulin, semilente, NPH, lente, protamine zinc insulin (PZI), ultralente, insuline glargine, insulin aspart, acylated insulin, monomeric insulin, superactive insulin, hepatoselective insulin, and any other insulin analog or derivative, and mixtures of any of the foregoing. Insulin that is suitable for use herein includes, but is not limited to, the insulin forms disclosed in U.S. Patent Nos. 4,992,417; 4,992,418; 5,474,978; 5,514,646; 5,504,188; 5,547,929; 5,650,486; 5,693,609; 5,700,662; 5,747,642; 5,922,675; 5,952,297; and 6,034,054; and published PCT applications WO 00/121197; WO 09/010645; and WO 90/12814. Insulin analogs include, but are not limited to, superactive insulin analogs, monomeric insulins, and hepatospecific insulin analogs.

Methods of treating schizophrenia

The present invention further provides methods of treating schizophrenia, the methods generally involving administering to an individual in need thereof an effective amount of a HERV polypeptide.

In these embodiments, an "effective amount" of a HERV polypeptide is an amount that, when administering to an individual in need thereof in one or more doses, reduces at least one symptom of schizophrenia by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or more, compared to the level or severity of the symptom in the individual in the absence of treatment with the HERV polypeptide. Symptoms of schizophrenia are known in the art, and include, e.g., "positive" symptoms (e.g., delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior); and "negative" symptoms (e.g, alogia, affective flattening, avolition).

FORMULATIONS

A HERV polypeptide, as described above, can be formulated in any of a variety of ways for administration to an individual in need thereof. The present invention provides pharmaceutical formulations comprising a HERV polypeptide. Immunogenic compositions comprising a HERV polypeptide are described above. Additional formulations are described below.

A subject formulation comprising a HERV polypeptide generally includes one or more of an excipient (e.g., sucrose, starch, mannitol, sorbitol, lactose, glucose, cellulose, talc, calcium phosphate or calcium carbonate), a binder (e.g., cellulose, methylcellulose, hydroxymethylcellulose, polypropylpyrrolidone, polyvinylprrolidone, gelatin, gum arabic, polyethyleneglycol, sucrose or starch), a disintegrator (e.g., starch, carboxymethylcellulose, hydroxypropylstarch, low substituted hydroxypropylcellulose, sodium bicarbonate, calcium phosphate or calcium citrate), a lubricant (e.g., magnesium stearate, light anhydrous silicic acid, talc or sodium lauryl sulfate), a flavoring agent (e.g., citric acid, menthol, glycine or orange powder), a preservative (e.g., sodium benzoate, sodium bisulfite, methylparaben or propylparaben), a stabilizer (e.g., citric acid, sodium citrate or acetic acid), a suspending agent (e.g., methylcellulose, polyvinylpyrrolidone or aluminum stearate), a dispersing agent (e.g., hydroxypropylmethylcellulose), a diluent (e.g., water), and base wax (e.g., cocoa butter, white petrolatum or polyethylene glycol).

Tablets comprising an active agent may be coated with a suitable film-forming agent, e.g., hydroxypropylmethyl cellulose, hydroxypropyl cellulose or ethyl cellulose, to which a

suitable excipient may optionally be added, e.g., a softener such as glycerol, propylene glycol, diethylphthalate, or glycerol triacetate; a filler such as sucrose, sorbitol, xylitol, glucose, or lactose; a colorant such as titanium hydroxide; and the like.

Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 17th edition, 1985. The composition or formulation to be administered will, in any event, contain a quantity of the agent adequate to achieve the desired state in the subject being treated. The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

In some embodiments, e.g., for use in inducing or enhancing an immune response to a lentivirus, a HERV polypeptide is formulated for vaginal delivery. A subject formulation for intravaginal administration is formulated as an intravaginal bioadhesive tablet, intravaginal bioadhesive microparticle, intravaginal cream, intravaginal lotion, intravaginal foam, intravaginal ointment, intravaginal paste, intravaginal solution, or intravaginal gel.

DOSAGES

The appropriate dosage of a HERV polypeptide that, when administered in one or multiple doses, has the desired effect (e.g., increases a T cell immune response to a lentivirus; increases an immune response to a cancer cell; reduces an autoimmune response; etc.), will vary, depending on various factors, but will generally be in the range of from about 1 μg to about 100 mg, e.g., from about 1 μg to about 5 μg, from about 5 μg to about 10 μg, from about 10 μg to about 25 μg, from about 25 μg to about 50 μg, from about 50 μg to about 100 μg, from about 100 μg to about 500 μg, from about 500 μg to about 1 mg, from about 1 mg to about 10 mg, from about 10 mg to about 50 mg, or from about 50 mg to about 100 mg, administered in one dose or divided into multiple doses.

In some embodiments, the amount of HERV polypeptide per dose is determined on a per body weight basis. For example, in some embodiments, a HERV polypeptide is administered in an amount of from about 0.5 mg/kg to about 100 mg/kg, e.g., from about 0.5 mg/kg to about 1 mg/kg, from about 1 mg/kg to about 2 mg/kg, from about 2 mg/kg to about 3 mg/kg, from about 3 mg/kg to about 5 mg/kg, from about 5 mg/kg to about 7 mg/kg, from

about 7 mg/kg to about 10 mg/kg, from about 10 mg/kg to about 15 mg/kg, from about 15 mg/kg to about 20 mg/kg, from about 20 mg/kg to about 25 mg/kg, from about 25 mg/kg to about 30 mg/kg, from about 30 mg/kg to about 40 mg/kg, from about 40 mg/kg to about 50 mg/kg per dose, from about 50 mg/kg to about 60 mg/kg, from about 60 mg/kg to about 70 mg/kg, from about 70 mg/kg to about 80 mg/kg, from about 80 mg/kg to about 90 mg/kg, or from about 90 mg/kg to about 100 mg/kg, or more than about 100 mg/kg.

Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

In some embodiments, multiple doses of a HERV polypeptide are administered. The frequency of administration of a HERV polypeptide can vary depending on any of a variety of factors, e.g., severity of the symptoms, etc. For example, in some embodiments, a HERV polypeptide is administered once per month, twice per month, three times per month, every other week (qow), once per week (qw), twice per week (biw), three times per week (tiw), four times per week, five times per week, six times per week, every other day (qod), daily (qd), twice a day (qid), or three times a day (tid).

The duration of administration of a HERV polypeptide, e.g., the period of time over which a HERV polypeptide is administered, can vary, depending on any of a variety of factors, e.g., patient response, etc. For example, a HERV polypeptide can be administered over a period of time ranging from about one day to about one week, from about two weeks to about four weeks, from about one month to about two months, from about two months to about four months, from about four months to about six months, from about six months to about eight months, from about eight months to about 1 year, from about 1 year to about 2 years, or from about 2 years to about 4 years, or more.

ROUTES OF ADMINISTRATION

Conventional and pharmaceutically acceptable routes of administration include intranasal, intramuscular, intratracheal, intratumoral, transdermal, subcutaneous, intradermal, topical application, intravenous, vaginal, nasal, and other parenteral routes of administration. Suitable routes of administration also include oral and rectal routes. Routes of administration may be combined, if desired, or adjusted depending upon the agent and/or the desired effect. The composition can be administered in a single dose or in multiple doses.

A subject HERV composition can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including

systemic or localized routes. In general, routes of administration contemplated by the invention include, but are not necessarily limited to, enteral, parenteral, or inhalational routes.

Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, vaginal, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, intratumoral, peritumoral, and intravenous routes, i.e., any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of the agent. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

A subject HERV composition can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (e.g., using a suppository) delivery.

A subject HERV composition can be delivered to mucosal tissue, e.g., to vaginal tissue, to rectal tissue, etc.

METHODS OF GENERATING HERV-SPECIFIC CTLS

The present invention provides methods of generating a population of HERV-specific $CD8^+$ T cells *in vitro*. The methods generally involve contacting a $CD8^+$ T cell, or a precursor thereof, with a HERV polypeptide in association with an antigen-presenting platform, where the contacting is performed *in vitro*. The methods are useful for generating a population of HERV polypeptide-specific $CD8^+$ T cells, which are in turn useful in methods of treating disorders such as lentivirus infection (e.g., HIV infection) and cancer.

In some embodiments, $CD8^+$ T cells are obtained from an individual, and are contacted *in vitro* with a HERV polypeptide in association with an antigen-presenting platform. In some embodiments, a mixed population of cells that comprises $CD8^+$ T cells is obtained from an individual; and $CD8^+$ T cells are isolated from the mixed population, generating an unstimulated $CD8^+$ T cell population. The unstimulated $CD8^+$ T cell population is then contacted *in vitro* a HERV polypeptide in association with an antigen-presenting platform. The contacting step activates at least a portion of the unstimulated $CD8^+$ T cell population to become specific for a HERV polypeptide.

The source of the mixed cell population that comprises a $CD8^+$ T cell can be, e.g., whole blood. The mixed cell population can manipulated in one or more ways or steps, e.g., to remove red blood cells; to select for $CD8^+$ T cells; and/or to select against $CD4^+$ T cells or other non-$CD8^+$ cell populations. The number of unstimulated $CD8^+$ cells can range from about 10^2 cells to about 10^9 cells, e.g., from about 10^2 cells to about 10^3 cells, from about 10^3

cells to about 10^4 cells, from about 10^4 cells to about 10^5 cells, from about 10^5 cells to about 5 x 10^5 cells, from about 5 x 10^5 cells to about 10^6 cells, from about 10^6 cells to about 5 x 10^6 cells, from about 5 x 10^6 cells to about 10^7 cells, from about 10^7 cells to about 5 x 10^7 cells, from about 5 x 10^7 cells to about 10^8 cells, from about 10^8 cells to about 5 x 10^8 cells, or from about 5 x 10^8 cells to about 10^9 cells.

The antigen-presenting platform can be an antigen-presenting cell (APC), e.g., an APC pulsed with a HERV peptide, where the APC can be live or can be inactivated. In some embodiments, the antigen-presenting platform is a bead (e.g., a plastic bead, a magnetic bead, etc.), or other particle, to which a HERV peptide is bound. Antigen-presenting platforms other than naturally-occurring APCs are known in the art and include, but are not limited to, beads; inactivated surface-engineered viruses (see, e.g., Mosca et al. (2007) *Retrovirol.* 4:32); artificial APCs, e.g., liposomes (see, e.g, U.S. Patent Publication No. 2006/0034865); and the like.

The antigen-presenting platform will include, in addition to a HERV peptide, one or more surface molecules sufficient for stimulating expansion of a HERV-specific $CD8^+$ T cell population, e.g., MHC class I molecules (e.g., HLA Class I molecules), etc. The antigen-presenting platform can also include one or more co-stimulatory molecules, where suitable co-stimulatory molecules include, but are not limited to, an anti-CD28 antibody, an anti-CD49d antibody, and the like).

The unstimulated $CD8^+$ T cells are contacted *in vitro* with a HERV peptide in association with an antigen-presenting platform; and the number of HERV-specific $CD8^+$ T cells is increased. The method results in a 10-fold to a 10^6-fold increase in the number of HERV-specific $CD8^+$ T cells. The number of HERV-specific $CD8^+$ cells obtained by a subject method can range from about 10^3 to about 10^9 cells, e.g., from about 10^3 cells to about 10^4 cells, from about 10^4 cells to about 10^5 cells, from about 10^5 cells to about 5 x 10^5 cells, from about 5 x 10^5 cells to about 10^6 cells, from about 10^6 cells to about 5 x 10^6 cells, from about 5 x 10^6 cells to about 10^7 cells, from about 10^7 cells to about 5 x 10^7 cells, from about 5 x 10^7 cells to about 10^8 cells, from about 10^8 cells to about 5 x 10^8 cells, or from about 5 x 10^8 cells to about 10^9 cells.

The present invention provides treatment methods using the HERV-specific $CD8^+$ T cells. In some embodiments, the methods are methods of treating an HIV infection. In other embodiments, the methods are methods of treating cancer. The methods generally involve administering to an individual in need thereof an effective amount of HERV-specific $CD8^+$ T cells. In some embodiments, the HERV-specific $CD8^+$ T cells are autologous, e.g., the HERV-

specific $CD8^+$ T cells are administered to the same individual from which the mixed cell population was obtained (i.e., the donor individual and the recipient individual are the same). In other embodiments, the HERV-specific $CD8^+$ T cells are allogeneic, e.g., the HERV-specific $CD8^+$ T cells are administered to an individual (a recipient individual) not genetically identical to the individual from which the mixed cell population was obtained (the donor individual).

In some embodiments, the HERV-specific $CD8^+$ T cells are administered to a recipient individual in an amount of from about 10^3 to about 10^9 cells, e.g., from about 10^3 cells to about 10^4 cells, from about 10^4 cells to about 10^5 cells, from about 10^5 cells to about 5×10^5 cells, from about 5×10^5 cells to about 10^6 cells, from about 10^6 cells to about 5×10^6 cells, from about 5×10^6 cells to about 10^7 cells, from about 10^7 cells to about 5×10^7 cells, from about 5×10^7 cells to about 10^8 cells, from about 10^8 cells to about 5×10^8 cells, or from about 5×10^8 cells to about 10^9 cells, in one or more doses.

DIAGNOSTIC METHODS

The present invention provides various diagnostic methods, which methods utilize a subject HERV polypeptide or a subject HERV composition. Subject diagnostic methods include methods for monitoring a patient's response to treatment; methods for staging a disease; and methods for detecting a disease.

In some embodiments, a subject diagnostic method involves detecting the presence in an individual of a cancer cell that produces a HERV polypeptide. Methods for detecting a cancer cell that produces a HERV polypeptide include immunological methods, e.g., use of an antibody specific for a HERV polypeptide, where immunological assays include, e.g., immunohistological assays, and fluorescence activated cell analysis assays (e.g., fluorescence activated cell sorting assays, using a fluorescently labeled antibody to a HERV polypeptide).

In other embodiments, a subject diagnostic method generally involves detecting the number of HERV-specific $CD8^+$ T cells in a biological sample obtained from an individual. The number of HERV-specific $CD8^+$ T cells can be determined using, e.g., a ^{51}Cr release assay, where target cells pulsed with a HERV peptide and labeled with ^{51}Cr are contacted with a test sample that may contain HERV-specific $CD8^+$ T cells. The number of HERV-specific $CD8^+$ T cells is determined by measuring release of ^{51}Cr from the target cells.

In other embodiments, a subject diagnostic method involves detecting a HERV polypeptide in the serum or plasma (or other biological fluid) of an individual. Detection of a HERV polypeptide in a biological fluid obtained from an individual can be carried out using, e.g., immunological assays employing antibody specific for a HERV polypeptide. Suitable

immunological assays include, but are not limited to, enzyme-linked immunosorbent assays (ELISA), radioimmunoassays (RIA), protein blot ("Western blot") assays, immunoprecipitation assays, and the like.

HERV-specific antibodies

As noted above, in some embodiments, a subject diagnostic assay will employ an antibody specific for a HERV polypeptide (an "anti-HERV antibody"). Suitable anti-HERV antibodies include whole antibody of any isotype; epitope-binding fragments of an anti-HERV antibody; polyclonal antibodies; monoclonal antibodies; artificial antibodies; single-chain antibodies; and the like.

Monoclonal antibodies are produced by conventional techniques. Generally, the spleen and/or lymph nodes of an immunized host animal provide a source of plasma cells. The plasma cells are immortalized by fusion with myeloma cells to produce hybridoma cells. Culture supernatant from individual hybridomas is screened using standard techniques to identify those producing antibodies with the desired specificity. Suitable animals for production of monoclonal antibodies include mouse, rat, hamster, guinea pig, rabbit, etc. The antibody may be purified from the hybridoma cell supernatants or ascites fluid by conventional techniques, *e.g.* affinity chromatography using protein bound to an insoluble support, protein A sepharose, *etc.*

The antibody may be produced as a single chain, instead of the normal multimeric structure. Single chain antibodies are described in Jost *et al.* (1994) J.B.C. 269:26267–73, and others. DNA sequences encoding the variable region of the heavy chain and the variable region of the light chain are ligated to a spacer encoding at least about 4 amino acids of small neutral amino acids, including glycine and/or serine. The protein encoded by this fusion allows assembly of a functional variable region that retains the specificity and affinity of the original antibody.

Suitable anti-HERV antibodies also include "artificial" antibodies, e.g., antibodies and antibody fragments produced and selected *in vitro*. In some embodiments, such antibodies are displayed on the surface of a bacteriophage or other viral particle. In many embodiments, such artificial antibodies are present as fusion proteins with a viral or bacteriophage structural protein, including, but not limited to, M13 gene III protein. Methods of producing such artificial antibodies are well known in the art. See, e.g., U.S. Patent Nos. 5,516,637; 5,223,409; 5,658,727; 5,667,988; 5,498,538; 5,403,484; 5,571,698; and 5,625,033.

Antibody fragments, such as Fv, $F(ab')_2$ and Fab may be prepared by cleavage of the intact protein, *e.g.* by protease or chemical cleavage. Alternatively, a truncated gene is

designed. For example, a chimeric gene encoding a portion of the $F(ab')_2$ fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

An anti-HERV antibody will in some embodiments be detectably labeled, e.g., with a radioisotope, an enzyme which generates a detectable product, a fluorescent protein, a chromogenic protein, and the like. An anti-HERV antibody may be further conjugated to other moieties, such as members of specific binding pairs, e.g., biotin (member of biotin-avidin specific binding pair), and the like. An anti-HERV antibody may also be bound to a solid support, including, but not limited to, polystyrene plates or beads, magnetic beads, test strips, membranes, and the like.

An antibody specific for a HERV polypeptide can be labeled, directly or indirectly. Direct labels include radioisotopes (e.g., ^{125}I; ^{35}S, and the like); enzymes whose products are detectable (e.g., luciferase, β-galactosidase, horse radish peroxidase, alkaline phosphatase, and the like); fluorescent labels (e.g., fluorescein isothiocyanate, rhodamine, phycoerythrin, and the like); fluorescence emitting metals, e.g., ^{152}Eu, or others of the lanthanide series, attached to the antibody through metal chelating groups such as EDTA; chemiluminescent compounds, e.g., luminol, isoluminol, acridinium salts, and the like; bioluminescent compounds, e.g., luciferin; fluorescent proteins (e.g., a green fluorescent protein, a yellow fluorescent protein, etc.); and the like. Indirect labels include second antibodies specific for HERV-specific antibodies, wherein the second antibody is labeled as described above; and members of specific binding pairs, e.g., biotin-avidin, and the like.

In some embodiments, an anti-HERV antibody comprises, covalently linked to the antibody, a protein that provides for a detectable signal. Suitable proteins include, but are not limited to, fluorescent proteins and enzymes (e.g., β-galactosidase, luciferase, horse radish peroxidase, alkaline phosphatase, etc.). Suitable fluorescent proteins include, but are not limited to, a green fluorescent protein (GFP), including, but not limited to, a GFP derived from *Aequoria victoria* or a derivative thereof, a number of which are commercially available; a GFP from a species such as *Renilla reniformis, Renilla mulleri,* or *Ptilosarcus guernyi*, as described in, e.g., WO 99/49019 and Peelle et al. (2001) *J. Protein Chem.* 20:507-519; any of a variety of fluorescent and colored proteins from Anthozoan species, as described in, e.g., Matz et al. (1999) *Nature Biotechnol.* 17:969-973, U.S. Patent Publication No. 2002/0197676, or U.S. Patent Publication No. 2005/0032085; and the like.

Monitoring patient response to treatment for a lentivirus infection

In some embodiments, a subject HERV polypeptide composition is useful for monitoring a patient's response to treatment for a lentivirus infection, e.g., an HIV infection. Thus, the present invention further provides methods for monitoring a patient's response to treatment for a lentivirus infection, e.g., an HIV infection. The methods generally involve contacting a white blood cell (WBC) from a patient *in vitro* with a subject HERV polypeptide; and detecting a cytokine secreted by the WBC in response to contact with the HERV polypeptide. A reduction in cytokine production by the WBC in response to contact with a HERV polypeptide is an indication that the treatment is effective in treating a lentivirus infection (e.g., in achieving a reduction in viral load, in achieving an increase in $CD4^+$ T lymphocyte levels (in the case of an HIV infection), and the like). Suitable WBC include, but are not limited to, peripheral blood mononuclear cells (PBMC), isolated T lymphocytes, isolated $CD4^+$ T lymphocytes, isolated $CD8^+$ T lymphocytes, natural killer (NK) cells, natural killer T lymphocytes (NKT, e.g., $NK1.1^+$ T lymphocytes), and the like.

HERV polypeptides suitable for use in a subject monitoring method can be 9 amino acids, 10 amino acids, 11 amino acids, 12 amino acids, 12-15 amino acids, 15-18 amino acids, 18-20 amino acids, or 20-25 amino acids long, or longer. Suitable HERV polypeptides include any of the HERV polypeptides discussed above. In some embodiments, the HERV polypeptide comprises an amino acid sequence as set forth in any one of SEQ ID NOs:1-25.

Cytokines that are secreted from PBMC and that are detected in a subject patient monitoring method include, but are not limited to, IFN-γ, TNF-α, and IL-2.

Methods for detecting secreted cytokines that are suitable for use in a subject patient monitoring method include, but are not limited to, immunological assays, e.g., enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), an enzyme-linked immunospot (ELISPOT) assay; cellular assays; and the like.

In some embodiments, a reduction of at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% or more, in cytokine production by WBC in response to contact with a HERV polypeptide indicates that the treatment for the lentivirus infection is efficacious.

Patient samples comprising WBC can be obtained before and after treatment; or at various times during the course of treatment, and the level of cytokine production compared between a sample taken at a first time point and a sample taken at a second (later) time point.

In some embodiments, PBMC obtained from a patient are contacted with one or more HERV polypeptides *in vitro*; and an ELISPOT assay is used to detect cytokine production. The ELISPOT assay has been described in the art. See, e.g., Lalvani et al. (1997) *J. Exp. Med.* 186:859; and U.S. Patent No. 5,853,697. In these embodiments, the level of cytokines produced by the PBMC is expressed as the number of spot-forming units (SFU) per 10^6 PBMC. A reduction in the number of SFU indicates that a treatment for a lentivirus infection is effective.

Monitoring patient response to cancer treatment

The present invention provides methods of monitoring patient response to a treatment regimen for cancer. The level of a HERV polypeptide associated with the cancer is monitored, before, during a treatment regimen, and after a treatment regimen.

In some embodiments, the level of a HERV polypeptide is monitored, e.g., in serum, on the surface of a particular cell population, etc.

Staging a disease

The present invention provides methods of staging a disease in an individual, where the level of a HERV polypeptide is associated with the stage or severity of the disease. The methods generally involve detecting the level of a HERV polypeptide in a biological sample obtained from the individual. The level of the HERV polypeptide in the biological sample is correlated with the severity of the disease or disorder, and used to stage the disease.

In some embodiments, a subject method of staging a disease involves detecting the number of $CD8^+$ T cells, in a biological sample obtained from an individual, that are specific for a subject HERV polypeptide. In some embodiments, the number of HERV-specific $CD8^+$ T cells is an indication of the stage of the disease.

Detecting a disease

The present invention provides methods of detecting a disease such as a cancer in an individual, where the presence or level of a HERV polypeptide in a biological sample obtained from the individual indicates the presence of a cancerous cell in the biological sample (and hence the individual). The methods generally involve detecting the level of a HERV polypeptide in a biological sample obtained from the individual. Where the level of the HERV polypeptide is higher than the level associated with a normal cell, such is an indication of the presence in the sample of a cancerous cell.

SUBJECTS SUITABLE FOR TREATMENT

Treatment of lentivirus infection

The methods of the present invention are suitable for treating individuals who have a lentiviral infection; uninfected individuals who are at risk of contracting a lentiviral infection; individuals who were treated for a lentiviral infection, but failed to respond to the treatment; and individuals who were treated for a lentiviral infection, but who relapsed.

For example, the methods of the present invention are suitable for treating individuals who have a human immunodeficiency virus (HIV) infection; individuals who are naïve with respect to HIV infection, but who at risk of contracting an HIV infection; and individuals who were treated for an HIV infection, but who either failed to respond to the treatment, or who initially responded to treatment but subsequently relapsed. Such individuals include, but are not limited to, uninfected individuals with healthy, intact immune systems, but who are at risk for becoming HIV infected ("at-risk" individuals). At-risk individuals include, but are not limited to, individuals who have a greater likelihood than the general population of becoming HIV infected. Individuals at risk for becoming HIV infected include, but are not limited to, individuals at risk for HIV infection due to sexual activity with HIV-infected individuals; intravenous drug users; individuals who may have been exposed to HIV-infected blood, blood products, or other HIV-contaminated body fluids; and babies who are being nursed by HIV-infected mothers. Individuals suitable for treatment include individuals infected with, or at risk of becoming infected with, HIV-1 and/or HIV-2 and/or HIV-3, or any variant thereof.

Treatment of HTLV infection

The above-described methods can be used to treat a human T cell leukemia virus (HTLV) infection in an individual, e.g, an HTLV-I or HTLV-II infection. Thus, a subject method is also suitable for treating individuals who have been infected with an HTLV; individuals who have not yet been infected with HTLV, but who are at risk of becoming infected with HTLV; and individuals who have not yet been infected with HTLV, but who may in the future become infected with HTLV.

Cancer treatment

The methods of the present invention are suitable for treating individuals diagnosed with a cancer associated with expression of HERV, where such cancers include, but are not limited to, breast cancer, ovarian cancer, melanoma, teratoma, seminoma, prostate cancer, and testicular cancer. The methods of the present invention are suitable for treating individuals who have been diagnosed with breast cancer; individuals who have been diagnosed with ovarian cancer; and individuals who have been diagnosed with testicular cancer. A subject

method of treating cancer is also suitable for treating individuals who have been treated for breast cancer, ovarian cancer, melanoma, teratoma, seminoma, prostate cancer, or testicular cancer, and who either failed to respond to the treatment, or responded initially, then relapsed.

Treatment of an autoimmune disorder

The methods of the present invention are suitable for treating individuals diagnosed with an autoimmune disorder, where such autoimmune disorders include, but are not limited to, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and Type 1 diabetes. The methods of the present invention are suitable for treating individuals who have been treated for an autoimmune disorder, and who either failed to respond to the treatment, or responded initially, then relapsed.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1: HERV peptides stimulate cytokine production in human PBMCs.

MATERIALS AND METHODS

Patients. HIV-1 positive volunteers were selected for this study. The study was approved by the local institutional review board and subjects were given written informed consent. Studies were performed on cryopreserved PBMC from various patient timepoints.

Peptide selection. Selection of candidate HERV epitopes was based on translated HERV protein sequence data compiled from publicly available databases. HIV-1 peptides were designed from the sequences of known HIV-1 epitopes listed in the Los Alamos National Laboratory HIV immunology database. Antigenic regions of HERV insertions were assigned

an HLA restriction with epitope prediction software [SYFPEITHI [29]; SEQ ID NO:36] or based on the HLA restriction of the corresponding HIV-1 epitope.

ELISPOT assay. ELISPOT analysis was performed as previously described[34]. Plates were incubated 15-18 hours at 37 °C. Equivalent antigen concentrations were used for HIV and HERV peptide response comparisons. Assays were performed with duplicate wells for each condition, expect where cell recovery from archived samples dictated the use of single wells. Plates were counted with an AID ELISPOT reader (Cell Technology,). Spot totals for duplicate wells were averaged, and all spot numbers were normalized to numbers of IFN-γ spot-forming units (SFU) per $1x10^6$ PBMC. Spot values from media control wells were subtracted to determine responses to each peptide. Any resulting peptide values <0 following media subtraction were set to 0 for further analysis.

HERV-K expression detection. Expression levels of a HERV-K derived envelope transcript[35] were measured in HIV-1 positive 1 ml plasma samples and HIV-1 negative low-risk controls. Plasma samples were centrifuged at low speed and filtered prior to RNA collection to remove remaining cellular contaminants. High speed centrifugation was used to pellet particles for RNA isolation with Trizol reagent (Invitrogen). Samples were pre-treated with DNAse to eliminate genomic DNA contamination as a source of amplified HERV sequences. RT-PCR was performed on samples along with control amplifications without RT enzyme. As a calibration standard, cellular transcript expression of HERV and the housekeeping control gene β-actin was measured in cDNA prepared from 2.5×10^6 HIV-negative donor PBMCs. Quantification standards were also prepared by serial dilution of the cellular cDNA. Quantitative PCR with primers specific for the transcripts of interest was performed on all samples with the ABI Prism 7900HT Sequence Detection System (Applied Biosystems) using SYBR-Green detection. Expression levels are presented as percentages relative to PBMC derived standards, and represent the means of triplicate reactions. Gel electrophoresis and melting point analysis of PCR products were used to confirm product purity and accurate amplicon size.

^{51}Cr release assays

Cryopreserved peripheral blood mononuclear cells (PBMC) from a study participant who responded to the HERV-L IQ10 peptide were stimulated for 7 days with peptides or pools of each antigen. Autologous, irradiated, peptide-pulsed feeder cells were used to restimulate after 7 days. Cells were tested for their ability to lyse peptide-pulsed, autologous, EBV-transformed B cell lines by measuring the percentage of specific ^{51}Cr release.

RESULTS

To identify differences between expression levels of HERVs in HIV-1 positive and negative subjects, an RT-PCR analysis was performed on plasma to quantify a transcript derived from the youngest family of endogenous retroviruses in the human genome, HERV-K (Figure 1A). Expression of the HERV-K transcript was detected in HIV-1 positive plasma, but not in HIV-1 negative controls. The amount of HERV-K transcripts in the plasma of HIV-1 positive individuals was greatly out of proportion to that of other non virion-associated cellular transcripts (β-actin), thus ruling out cellular debris as an etiology for these transcripts. Data from additional individuals are presented in Figure 1B, which shows plasma RNA levels of HERV-K in HIV-1-positive and HIV-1-negative individuals' plasma.

Figures 1A and 1B. Expression of HERV-K transcripts in HIV positive and negative individuals' plasma. a, Levels of a HERV-K transcript derived from the envelope region measured relative to levels detected in peripheral blood cells (set to a value of 100 for comparison) shown as unfilled bars. Levels of a cellular control gene (β-actin) are shown as filled bars. Levels of the control gene measured in peripheral blood cells were also set to 100 for relative comparison to other samples. b, Levels of the HERV-K transcript measured in the plasma of HIV-1 positive (filled circles) and HIV-1-negative individuals (open circles).

When HERVs are expressed, the potential exists to generate an immune response against these antigens. Given that these are also endogenous antigens, it is unclear whether the response will be immunogenic or tolerogenic in nature. It was hypothesized that in regions of HIV-1 that are highly similar to HERVs, tolerance to HERVs could impair the HIV-1-specific immune response. Cross-tolerance has recently been suggested as a mechanism hampering the body's ability to produce antibodies that neutralize HIV-1 due to their cross-reactivity with a self-antigen cardiolipin[18]. Although HIV-1 and endogenous retroviruses are phylogenetically distant[19], the similarity between them was analyzed from the perspective of a T cell receptor, focusing on short regions of high similarity corresponding to the length of T cell epitopes (8-12 amino acids). These regions of similarity are typically rejected in standard phylogenetic analysis, as they are small enough to occur frequently by chance, without indicating any genetic relatedness. Because the T cell recognizes proteins in short peptides presented on HLA molecules, these regions of similarity have significance for the immune response (Figure 2). Since reverse transcriptase is a highly conserved protein, we expected and observed both clustered and distributed amino acid identity. Less conserved proteins such as Gag showed primarily clustered amino acid identities.

Figure 2. HERV/HIV amino acid alignments of HIV HXB-2 and various HERV insertions (identified by their HERVd[28] or NCBI accession number) showing segments of the Gag and Reverse Transcriptase proteins. Identical amino acids are shown in boxes. Alignments were anchored based on short regions of similarity identified with BLAST[36] short nearly exact match search settings, which included both amino acid similarity (not shown in this figure) and identity.

Thirty-one HIV-1 positive volunteers and five low-risk HIV-1 negative controls were screened by ELISPOT for responses to a panel of peptides derived from HERV insertions and HIV-1 proteins (Table 1) with varying levels of amino acid sequence identity to each other.

Table 1. HERV and HIV-1-derived peptide data.

Peptide Name	Epitope Alignment	Percent Identity	Sequence Identifier	HLA restriction	Start Position	Accession	Sequence Origin
not tested	KEALLDTGAD | | ||||	70%	SEQ ID NO:39	No known epitope	Protease: 20		HXB-2
HERV-K FD10	FEGLVDTGAD		SEQ ID NO:12			gi52001472	HERV-K
HERV Gag KK9	KIRLRPGGK |||| ||	67%	SEQ ID NO:40	A3/B27	Gag p17: 18		HXB-2
HERV-L KF9	KIRLPPGYF		SEQ ID NO:8			162613	HERV-L
not tested	SSGRMIMEK ||| | ||	67%	SEQ ID NO:41	No known epitope	gp160: 143		HXB-2
HERV-L SF9	SSGLMLMEF		SEQ ID NO:7			162605	HERV-L
HIV RT TI9	TAFTIPSI ||||| |	67%	SEQ ID NO:42	B51	RT p51: 128		HXB-2
HERV-K FI8	FAFTIPAI		SEQ ID NO:10			gi5802821	HERV-K
HIV RT IL9	IPLTEEAEL ||| |	44%	SEQ ID NO:43	B35	RT p51: 293		HXB-2
HERV-K VR9	VPLTKEQVR		SEQ ID NO:14				HERV-K
HIV Gag SL9	SLYNTVATL | |||	44%	SEQ ID NO:44	A2	Gag p17: 77		HXB-2
HERV-L PL9	PMVSTPATL		SEQ ID NO:4			162568	HERV-L
HIV gp160 SY9	SFEPIPIHY ||||	44%	SEQ ID NO:45		gp160: 209		HXB-2
HERV-C TE9	TLEPIPPGE		SEQ ID NO:16				HERV-C
not tested	LLQLTVWGI || | |	44%	SEQ ID NO:46	A2	gp160: 565		HXB-2
HERV-K FI9	FLQFKTWWI		SEQ ID NO:13			Rakoff-Nahoum	HERV-K

Peptide Name	Epitope Alignment	Percent Identity	Sequence Identifier	HLA restriction	Start Position	Accession	Sequence Origin
not tested	EIQKQGQGQ		SEQ ID NO:47	No known epitope	RT p51: 328		HXB-2
	| |||	44%					
HERV-K GQ9	GIPYNSQGQ		SEQ ID NO:11			gi67782351	HERV-K
HIV Nef LG13	LSHFLKEKGGLEG		SEQ ID NO:48	B35	Nef: 87		HXB-2
	| |||||	46%					
HERV-H LI12	LDLLTAEKGGLCI		SEQ ID NO:15				HERV-H
HIV RT VL9	VIYQYMDDL		SEQ ID NO:49	A2	RT p51: 179		HXB2
	| ||	33%					
HERV-L II9	ILVHYIDDI		SEQ ID NO:2			162563	HERV-L
HIV RT NY9	NPDIVIYQY		SEQ ID NO:50	B35	RT p51: 175		HXB-2
	|| |	33%					
HERV-L LY9	LQDIILVHY		SEQ ID NO:3			162563	HERV-L
not tested	QNIQGQMVHQ		SEQ ID NO:51	multiple	Gag p24: 4		HXB-2
	| ||	33%					
HERV-W DK10	DSIEGQLILK		SEQ ID NO:9			gi52000737	HERV-W
HIV RT TY9	TVLDVGDAY		SEQ ID NO:52	B35	RT p51: 107		HXB2
	| |	22%					
HERV-L AF9	AAIDLANAF		SEQ ID NO:5			162604	HERV-L
HIV RT VY10	VPLDEDFRKY		SEQ ID NO:53	B35	RT p51: 118		HXB2
	| |	20%					
HERV-L IQ10	IPVHKAHKKQ		SEQ ID NO:6			162604	HERV-L
HIV RT RG10	RYQYNVLPQG		SEQ ID NO:54	B51	RT p51: 143		HXB-2
	|	11%					
HERV-L SL10	SQGYINSPAL		SEQ ID NO:1			162563	HERV-L

Strong interferon gamma specific T cell responses were detected to HERV peptides in HIV-1 infected volunteers but not in HIV-1 negative controls (Figure 3, Mann-Whitney, P <0.05). The magnitude of the HIV-1 T cell response was directly associated with the magnitude of the HERV T cell response.

Figure 3. T cell responses to HERV and HIV-1 antigens in 29 HIV-1 positive and 13 low-risk HIV-1 negative individuals measured by Interferon-gamma ELISPOT. HERV peptides were grouped according to their similarity to HIV-1 peptide sequence, with 'Unique HERV Peptides' having 3 or less amino acids in common with an HIV-1 peptide, and 'HERV Peptides similar to HIV-1' having 4 or more peptides in common with HIV-1. Subsets of peptides were tested in each patient, with the number tested (n=6-23) varying depending on HLA type. Values shown for responses are normalized per peptide within each grouping (i.e. the sum of the response values to all peptides tested divided by the number of peptides tested for each patient). Responses in HIV-1 positive individuals are shown as closed circles and in HIV-1 negative individuals are shown as open circles. Responses to all HERV peptides were measured for HCV+ individuals and are shown as filled triangles. P-values are derived from the Mann-Whitney test.

As this association could indicate HIV-1 specific T cells cross-reacting on HERV antigens, the frequency of responses for each HERV peptide and its counterpart HIV-1 peptide was compared. For each HIV-1/HERV peptide pairing, there were variable numbers of amino acids in common between the two peptides. High frequency HERV peptide responses were detected at low levels of amino acid identity to HIV-1 peptides, indicating that HERV-specific responses are generated independently. It was concluded that cross-reactive HIV-1-specific T cells cannot be solely responsible for the responses against HERVs observed.

Figure 4 depicts an inverse correlation between anti-HERV T cell responses and HIV-1 plasma viral load. PBMC from twenty HIV-1+ individuals not on treatment were analyzed by ELISPOT for HERV responses. The mean response (>50 SFU/million PBMC) values for all HERV peptides tested had a significant inverse correlation to HIV-1 plasma viral load (Spearman, two-tailed, r=−0.49, P=0.03) and by linear regression (r^2=0.39, P=0.003) as shown in the figure.

Because the ability to control viral load by eliminating infected cells depends on killing, the ability of HERV specific CD8+ T cells to kill autologous B cells presenting their target peptide was measured. PBMC from one subject (OP841) were peptide stimulated to enrich for responsive $CD8^+$ T cells. After a two-week peptide stimulation, the ^{51}Cr-release assay was used to measure the ability of the enriched $CD8^+$ T cells to kill EBV-transformed B

cell targets presenting cognate peptide. $CD8^+$ T cells enriched by stimulation with HERV peptide were able to kill B cell targets presenting their cognate peptide but did not lyse targets loaded with a non-cognate or no peptide (Figure 5).

Figure 5 depicts ^{51}Cr release from target cells. HERV-L IQ10-specific T cells were tested against autologous B cells pulsed with HERV-L IQ10 peptide (filled circles), control peptide (open circles) or no peptide (filled triangles).

The data demonstrate an elevation in HERV transcript expression and T cell responses directed at HERV peptides associated with HIV-1 infection. A naturally-arising T cell response against HERVs in HIV-1-infected individuals indicates the feasibility of inducing responses earlier in infection, or in at risk uninfected individuals, as a novel HIV-1 vaccine paradigm. One of the greatest difficulties in HIV-1 vaccine development is overcoming the mutability of the virus, which enables it to evade specific immune responses elicited with a vaccine. HERVs are genome-encoded elements; translation products produced from de-regulated transcription of HERV insertions is expected to be far less variable than HIV-1 proteins. If HERV antigen production and presentation is a consequence of HIV-1 infection of a cell, the HERV products serve as a stably recognizable surrogate marker signalling HIV-1 infection to the immune system. Educating the immune system to recognize the HERV surrogate marker through vaccination induces killing of HIV-1-infected cells, circumventing the need to recognize highly variable HIV-1 antigens.

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While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. An immunogenic composition comprising a human endogenous retrovirus (HERV) polypeptide and a pharmaceutically acceptable carrier.

2. The immunogenic composition of claim 1, wherein the HERV polypeptide comprises an amino acid sequence as set forth in any one of SEQ ID NOs:1-25.

3. The immunogenic composition of claim 1, wherein the composition is formulated for parenteral administration.

4. The immunogenic composition of claim 1, wherein the composition is formulated for administration to a mucosal tissue.

5. The immunogenic composition of claim 1, further comprising an adjuvant.

6. The immunogenic composition of claim 5, wherein the adjuvant comprises aluminum hydroxide, MF59, or monophosphoryl lipidA.

7. An immunogenic composition comprising a nucleic acid comprising a nucleotide sequence encoding a human endogenous retrovirus (HERV) polypeptide.

8. The immunogenic composition of claim 7, wherein the HERV polypeptide comprises an amino acid sequence as set forth in any one of SEQ ID NOs:1-25.

9. The immunogenic composition of claim 7, wherein the composition is formulated for parenteral administration.

10. The immunogenic composition of claim 7, wherein the composition is formulated for administration to a mucosal tissue.

11. The immunogenic composition of claim 7, wherein the nucleic acid is a recombinant vector.

12. The immunogenic composition of claim 11, wherein the recombinant vector is a recombinant viral vector.

13. A method of inducing a T lymphocyte response in an individual to a host cell infected with a pathogenic virus, the method comprising administering to the individual the immunogenic composition of claim 1 or claim 7.

14. The method of claim 13, wherein the T lymphocyte response comprises a $CD8^+$ T cell response or a $CD4^+$ T cell response.

15. The method of claim 13, wherein the T lymphocyte response comprises a mucosal T lymphocyte response.

16. The method of claim 13, wherein the pathogenic virus is a human immunodeficiency virus.

17. The method of claim 13, wherein the individual has not been infected with the pathogenic virus.

18. The method of claim 13, wherein the individual has been infected with the pathogenic virus.

19. A method of inducing a T lymphocyte response in an individual to a cancer cell having HERV expression and displaying HERV epitopes on the surface of the cancer cell, the method comprising administering to the individual the immunogenic composition of claim 1 or claim 7.

20. An isolated human endogenous retrovirus (HERV) polypeptide.

21. A composition comprising an isolated human endogenous retrovirus (HERV) polypeptide.

22. A method of generating a population of $CD8^+$ T cells specific for a human endogenous retrovirus (HERV) peptide, the method comprising contacting a population of unstimulated $CD8^+$ T cells *in vitro* with a HERV peptide in association with an antigen-presenting platform, wherein said contacting provides for production of a population of HERV peptide-specific $CD8^+$ T cells.

SEQUENCE LISTING

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<211> 35
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<213> H. sapiens

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Tyr Thr His Asp Arg Ala Gln Ala Val Pro Glu Gly Thr Ser Lys Leu
1 5 10 15
His Glu Glu Val Ala Gln Met Pro Met Val Ser Thr Pro Ala Thr Leu

20 25 30
Ser Leu Pro
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<210> 31
<211> 179
<212> PRT
<213> H. sapiens

<400> 31
Ser Pro Trp Asn Ser Pro Val Phe Val Ile Gln Lys Lys Ser Gly Lys
1 5 10 15
Trp Arg Met Leu Thr Asp Leu Arg Ala Val Asn Ala Val Ile Gln Pro
20 25 30
Met Gly Pro Leu Gln Pro Gly Leu Pro Ser Pro Ala Met Ile Pro Lys
35 40 45
Asp Trp Pro Leu Ile Ile Ile Asp Leu Lys Asp Cys Phe Phe Thr Ile
50 55 60
Pro Leu Ala Glu Gln Asp Cys Glu Lys Phe Ala Phe Thr Ile Pro Ala
65 70 75 80
Ile Asn Asn Lys Glu Pro Ala Thr Arg Phe Gln Trp Lys Val Leu Pro
85 90 95
Gln Gly Met Leu Asn Ser Pro Thr Ile Cys Gln Thr Phe Val Gly Arg
100 105 110
Ala Leu Gln Pro Val Arg Glu Lys Phe Ser Asp Cys Tyr Ile Ile His
115 120 125
Cys Ile Asp Asp Ile Leu Cys Ala Ala Glu Thr Lys Asp Lys Leu Ile
130 135 140
Asp Cys Tyr Thr Phe Leu Gln Ala Glu Val Ala Asn Ala Gly Leu Ala
145 150 155 160
Ile Ala Ser Asp Lys Ile Gln Thr Ser Thr Pro Phe His Tyr Leu Gly
165 170 175
Met Gln Ile

<210> 32
<211> 109
<212> PRT
<213> H. sapiens

<400> 32
Arg Met Ile Val Asp Tyr Arg Lys Leu Asn Lys Gly Ser Thr Pro Thr
1 5 10 15
Ala Ala Ala Val Ser Asp Val Val Ser Leu Leu Glu Gln Ile Asn Thr
20 25 30
Ser Pro Asp Thr Trp Tyr Val Ala Thr Asp Leu Ala Asn Ala Phe Cys
35 40 45
Ser Ile Pro Val His Lys Ala His Gln Lys Gln Phe Ala Phe Gly Trp
50 55 60
Gln Gly Gln Glu Tyr Thr Phe Thr Val Leu Ser Gln Gly Tyr Ile Asn
65 70 75 80
Ser Pro Ala Leu Cys His Asn Leu Val Gln Arg Asp Leu Asp His Phe
85 90 95
Ser Leu Pro Gln Asp Ile Thr Leu Phe His Tyr Ile Asp
100 105

<210> 33
<211> 327
<212> DNA

<213> H. sapiens

<400> 33
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tcagatgtag tttcattgct tgagcaaatt aacacatctc ctgatacctg gtatgtggcc 120
actgacttgg caaatgcctt ttgctccatt cctgtccata aggcccacca gaagcaattt 180
gcatttggct ggcaaggcca ggaatatacc ttcactgtcc tatctcaggg gtatatcaac 240
tctccagctt tgtgtcataa tcttgttcag agagatcttg atcacttttc acttccacaa 300
gatatcacac tattccatta cattgat 327

<210> 34
<211> 584
<212> PRT
<213> H. sapiens

<400> 34
Met Ile Phe Ala Gly Lys Ala Pro Ser Asn Thr Ser Thr Leu Met Lys
1 5 10 15
Phe Tyr Ser Leu Leu Leu Tyr Ser Leu Leu Phe Ser Phe Pro Phe Leu
20 25 30
Cys His Pro Leu Pro Leu Pro Ser Tyr Leu His His Thr Ile Asn Leu
35 40 45
Thr His Ser Leu Leu Ala Ala Ser Asn Pro Ser Leu Val Asn Asn Cys
50 55 60
Trp Leu Cys Ile Ser Leu Ser Ser Ser Ala Tyr Thr Ala Val Pro Ala
65 70 75 80
Val Gln Thr Asp Trp Ala Thr Ser Pro Ile Ser Leu His Leu Arg Thr
85 90 95
Ser Phe Asn Ser Pro His Leu Tyr Pro Pro Glu Glu Leu Ile Tyr Phe
100 105 110
Leu Asp Arg Ser Ser Lys Thr Ser Pro Asp Ile Ser His Gln Gln Ala
115 120 125
Ala Ala Leu Leu Arg Thr Tyr Leu Lys Asn Leu Ser Pro Tyr Ile Asn
130 135 140
Ser Thr Pro Pro Ile Phe Gly Pro Leu Thr Thr Gln Thr Thr Ile Pro
145 150 155 160
Val Ala Ala Pro Leu Cys Ile Ser Trp Gln Arg Pro Thr Gly Ile Pro
165 170 175
Leu Gly Asn Leu Ser Pro Ser Arg Cys Ser Phe Thr Leu His Leu Arg
180 185 190
Ser Pro Thr Thr Asn Ile Asn Glu Thr Ile Gly Ala Phe Gln Leu His
195 200 205
Ile Thr Asp Lys Pro Ser Ile Asn Thr Asp Lys Leu Lys Asn Ile Ser
210 215 220
Ser Asn Tyr Cys Leu Gly Arg His Leu Pro Cys Ile Ser Leu His Pro
225 230 235 240
Trp Leu Ser Ser Pro Cys Ser Ser Asp Ser Pro Pro Arg Pro Ser Ser
245 250 255
Cys Leu Leu Ile Pro Ser Pro Glu Asn Asn Ser Glu Arg Leu Leu Val
260 265 270
Asp Thr Arg Arg Phe Leu Ile His His Glu Asn Arg Thr Phe Pro Ser
275 280 285
Thr Gln Leu Pro His Gln Ser Pro Leu Gln Pro Leu Thr Ala Ala Ala
290 295 300
Leu Ala Gly Ser Leu Gly Val Trp Val Gln Asp Thr Pro Phe Ser Thr
305 310 315 320
Pro Ser His Leu Phe Thr Leu His Leu Gln Phe Cys Leu Ala Gln Gly
325 330 335
Leu Phe Phe Leu Cys Gly Ser Ser Thr Tyr Met Cys Leu Pro Ala Asn
340 345 350
Trp Thr Gly Thr Cys Thr Leu Val Phe Leu Thr Pro Lys Ile Gln Phe
355 360 365

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Ala Asn Gly Thr Glu Glu Leu Pro Val Pro Leu Met Thr Pro Thr Gln
    370                 375                 380
Gln Lys Arg Val Ile Pro Leu Ile Pro Leu Met Val Gly Leu Gly Leu
385                 390                 395                 400
Ser Ala Ser Thr Val Ala Leu Gly Thr Gly Ile Ala Gly Ile Ser Thr
                405                 410                 415
Ser Val Met Thr Phe Arg Ser Leu Ser Asn Asp Phe Ser Ala Ser Ile
            420                 425                 430
Thr Asp Ile Ser Gln Thr Leu Ser Val Leu Gln Ala Gln Val Asp Ser
        435                 440                 445
Leu Ala Ala Val Val Leu Gln Asn Arg Arg Gly Leu Asp Leu Leu Thr
    450                 455                 460
Ala Glu Lys Gly Gly Leu Cys Ile Phe Leu Asn Glu Glu Cys Cys Phe
465                 470                 475                 480
Tyr Leu Asn Gln Ser Gly Leu Val Tyr Asp Asn Ile Lys Lys Leu Lys
                485                 490                 495
Asp Arg Ala Gln Lys Leu Ala Asn Gln Ala Ser Asn Tyr Ala Glu Pro
            500                 505                 510
Pro Trp Ala Leu Ser Asn Trp Met Ser Trp Val Leu Pro Ile Val Ser
        515                 520                 525
Pro Leu Ile Pro Ile Phe Leu Leu Leu Leu Phe Gly Pro Cys Ile Phe
    530                 535                 540
Arg Leu Val Ser Gln Phe Ile Gln Asn Arg Ile Gln Ala Ile Thr Asn
545                 550                 555                 560
His Ser Ile Arg Gln Met Phe Leu Leu Thr Ser Pro Gln Tyr His Pro
                565                 570                 575
Leu Pro Gln Asp Leu Pro Ser Ala
            580
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<212> DNA
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tatctccacc acactatcaa ccttacccat tctctcctag ccgcttctaa tccctcctta 180
gtgaacaact gctggctttg catttccctt tcttccagtg cctacacagc tgtccccgcc 240
ttacagacag actgggcaac atctcccatc tccctacacc tccgaacttc ctttaacagc 300
cctcaccttt accctcctga agaactcatt tactttctag acaggtccag caagacttcc 360
ccagacattt cacatcagca agctgccgcc ctccttcgca cttatttaaa aaacctttct 420
ccttatatca actctactcc ccccatatta ggacctctca caacacaaac tactattcct 480
gtggccgctc ctttgtgtat ctcttggcaa agacccactg gaattcccct aggtaatctt 540
tcaccttctc gatgttcctt tactcttcat ctccgaagtc caactacaaa catcaatgaa 600
acaattggag ccttccagct ccatattaca gacaagccct ctatcaatac tgacaaactt 660
aaaaacatta gcagtaatta ttgcttagga agacacttgc cctgtatttc actccatcct 720
tggctatctt ccccttgctc atcagactct cctcccaggc cctcttcttg tttacttata 780
cccagccccg aaaataacag tgaaagattg ctcgtagata ctcgacgttt tctcatacac 840
catgaaaatc gaaccttccc ctctacgcag ttaccccatc agtccccatt acaacctctg 900
acagctgccg ccctagctgg atccctagga gtctgggtac aagacacccc tttcagcact 960
ccttctcacc tttttacttt acatctccag ttttgcctcg cacaaggtct cttcttcctc 1020
tgtggatcct ctacctacat gtgcctacct gccaattgga caggcacatg tacactagtc 1080
ttccttaccc ccaaaattca atttgcaaat gggaccgaag agctccctgt tcccctcatg 1140
acaccgacac aacaaaaaag agttattcca ctaattcccc tgatggtcgg tttaggactt 1200
tctgcctcca ctgttgctct cggtactgga atagcaggca tttcaacgtc tgtcatgacc 1260
ttccgtagcc tgtctaatga cttctctgct agcatcacag acatatcaca aactttatca 1320
gtcctccagg cccaagttga ctctttagct gcagttgtcc tccaaaaccg ccgaggcctt 1380
gacttactca ctgctgaaaa aggaggactc tgcatatttct taaatgagga gtgttgtttt 1440
tacctaaatc aatctggcct ggtgtatgac aacattaaaa aactcaagga tagagcccaa 1500
aaacttgcca accaagcaag taattacgct gaacccccct gggcactctc taattggatg 1560

tcctgggtcc tcccaattgt tagtccttta atacccattt ttctccttct tttatttgga 1620
ccttgtatct tccgtttagt ttctcaattc atccaaaacc gtatccaggc catcaccaat 1680
cattctatac gacaaatgtt tcttctaaca tccccacaat atcacccctt accacaagac 1740
ctcccttcag cttaa 1755

<210> 36
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide

<400> 36
Ser Tyr Phe Pro Glu Ile Thr His Ile
1 5

<210> 37
<211> 87
<212> PRT
<213> Human immunodeficiency virus

<400> 37
Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu
1 5 10 15
Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu
20 25 30
Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys
35 40 45
Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro
50 55 60
Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp
65 70 75 80
Leu Tyr Val Gly Ser Asp Leu
85

<210> 38
<211> 88

<212> PRT
<213> Human immunodeficiency virus

<400> 38
Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp
1 5 10 15
Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys
20 25 30
His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro
35 40 45
Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu
50 55 60
Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn
65 70 75 80
Thr Val Ala Thr Leu Tyr Cys Val
85

<210> 39
<211> 10
<212> PRT

<213> Artificial Sequence

<220>
<223> synthetic peptide

<400> 39
Lys Glu Ala Leu Leu Asp Thr Gly Ala Asp
1 5 10

<210> 40
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide

<400> 40
Lys Ile Arg Leu Arg Pro Gly Gly Lys
1 5

<210> 41
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<212> PRT
<213> Artificial Sequence

<220>
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Ser Ser Gly Arg Met Ile Met Glu Lys
1 5

<210> 42
<211> 8
<212> PRT
<213> Human immunodeficiency virus

<400> 42
Thr Ala Phe Thr Ile Pro Ser Ile
1 5

<210> 43
<211> 9
<212> PRT
<213> Human immunodeficiency virus

<400> 43
Ile Pro Leu Thr Glu Glu Ala Glu Leu
1 5

<210> 44
<211> 9
<212> PRT
<213> Human immunodeficiency virus

<400> 44

Ser Leu Tyr Asn Thr Val Ala Thr Leu
1 5

<210> 45
<211> 9
<212> PRT
<213> Human immunodeficiency virus

<400> 45
Ser Phe Glu Pro Ile Pro Ile His Tyr
1 5

<210> 46
<211> 9
<212> PRT
<213> Human immunodeficiency virus

<400> 46
Leu Leu Gln Leu Thr Val Trp Gly Ile
1 5

<210> 47
<211> 9
<212> PRT
<213> Human immunodeficiency virus

<400> 47
Glu Ile Gln Lys Gln Gly Gln Gly Gln
1 5

<210> 48
<211> 13
<212> PRT
<213> Human immunodeficiency virus

<400> 48
Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly
1 5 10

<210> 49
<211> 9
<212> PRT
<213> Human immunodeficiency virus

<400> 49
Val Ile Tyr Gln Tyr Met Asp Asp Leu
1 5

<210> 50
<211> 9
<212> PRT
<213> Human immunodeficiency virus

<400> 50
Asn Pro Asp Ile Val Ile Tyr Gln Tyr
1 5

<210> 51
<211> 10
<212> PRT
<213> Human immunodeficiency virus

<400> 51
Gln Asn Ile Gln Gly Gln Met Val His Gln
1 5 10

<210> 52
<211> 9
<212> PRT
<213> Human immunodeficiency virus

<400> 52
Thr Val Leu Asp Val Gly Asp Ala Tyr
1 5

<210> 53
<211> 10
<212> PRT
<213> Human immunodeficiency virus

<400> 53
Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr
1 5 10

<210> 54
<211> 10
<212> PRT
<213> Human immunodeficiency virus

<400> 54
Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly
1 5 10

FIG. 1A

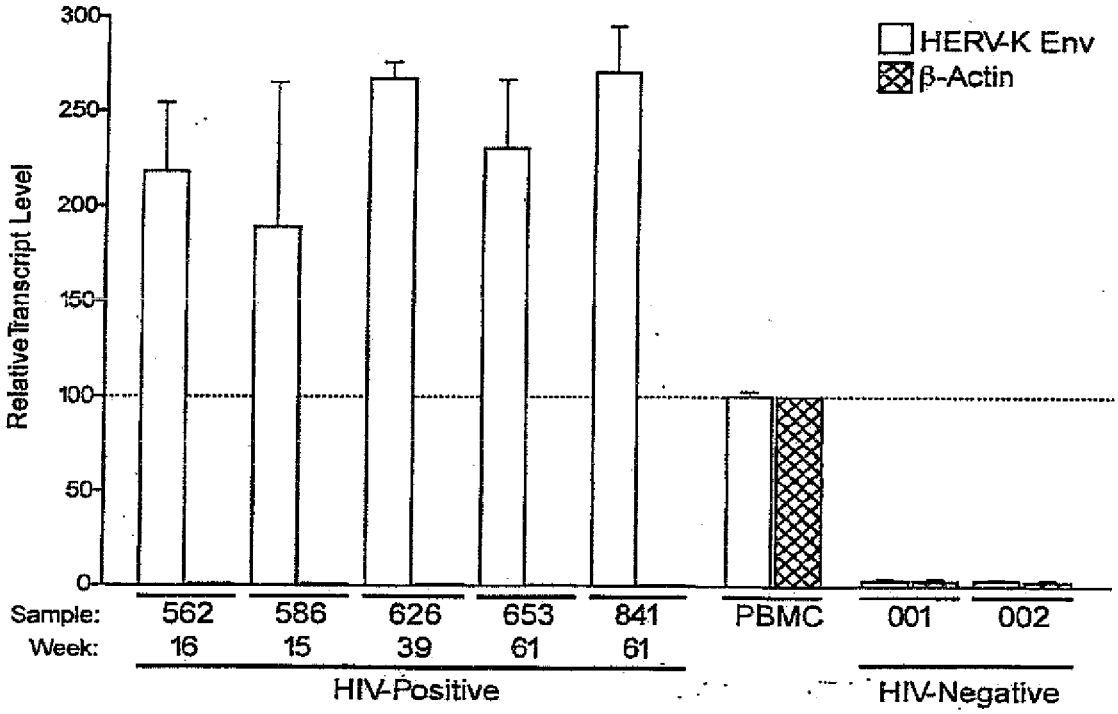

HERV-K Env
β-Actin
Relative Transcript Level
300
250
200
150
100
50
0
Sample:
Week:
562
16
586
15
626
39
653
61
841
61
PBMC
001
002
HIV-Positive
HIV-Negative

FIG. 1B

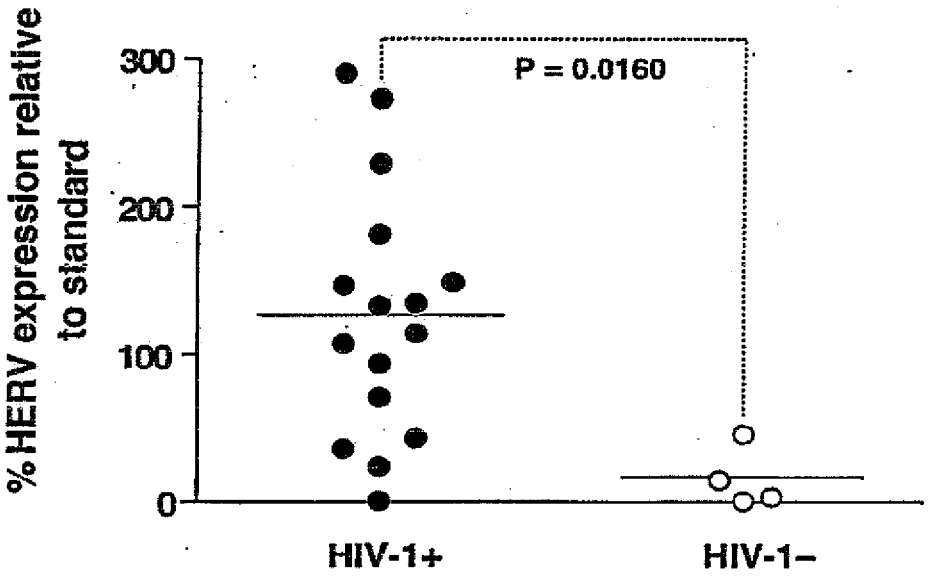
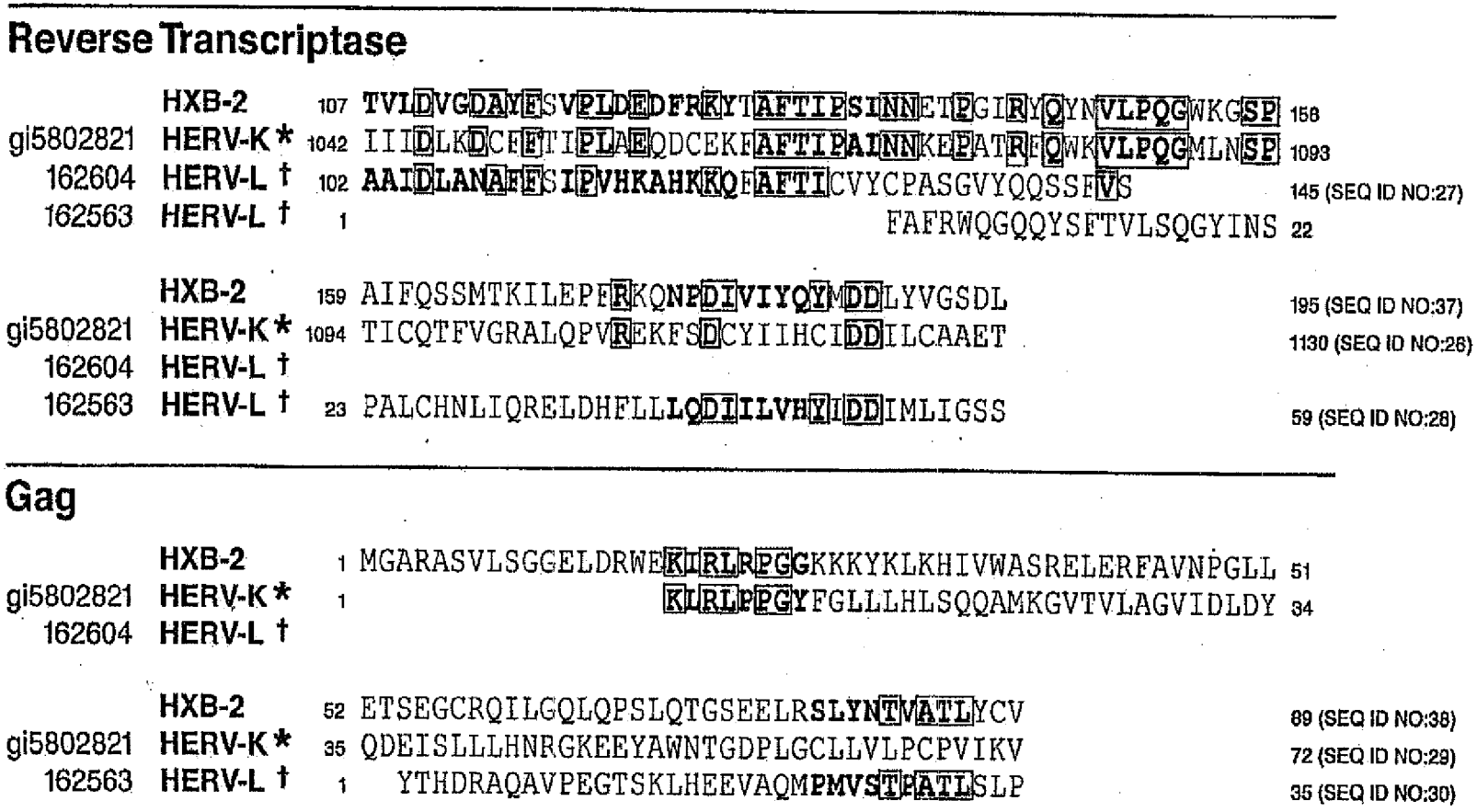

% HERV expression relative to standard
300
200
100
0
P = 0.0160
HIV-1+
HIV-1−

FIG. 2



*NCBI

†HERVdb

FIG. 3

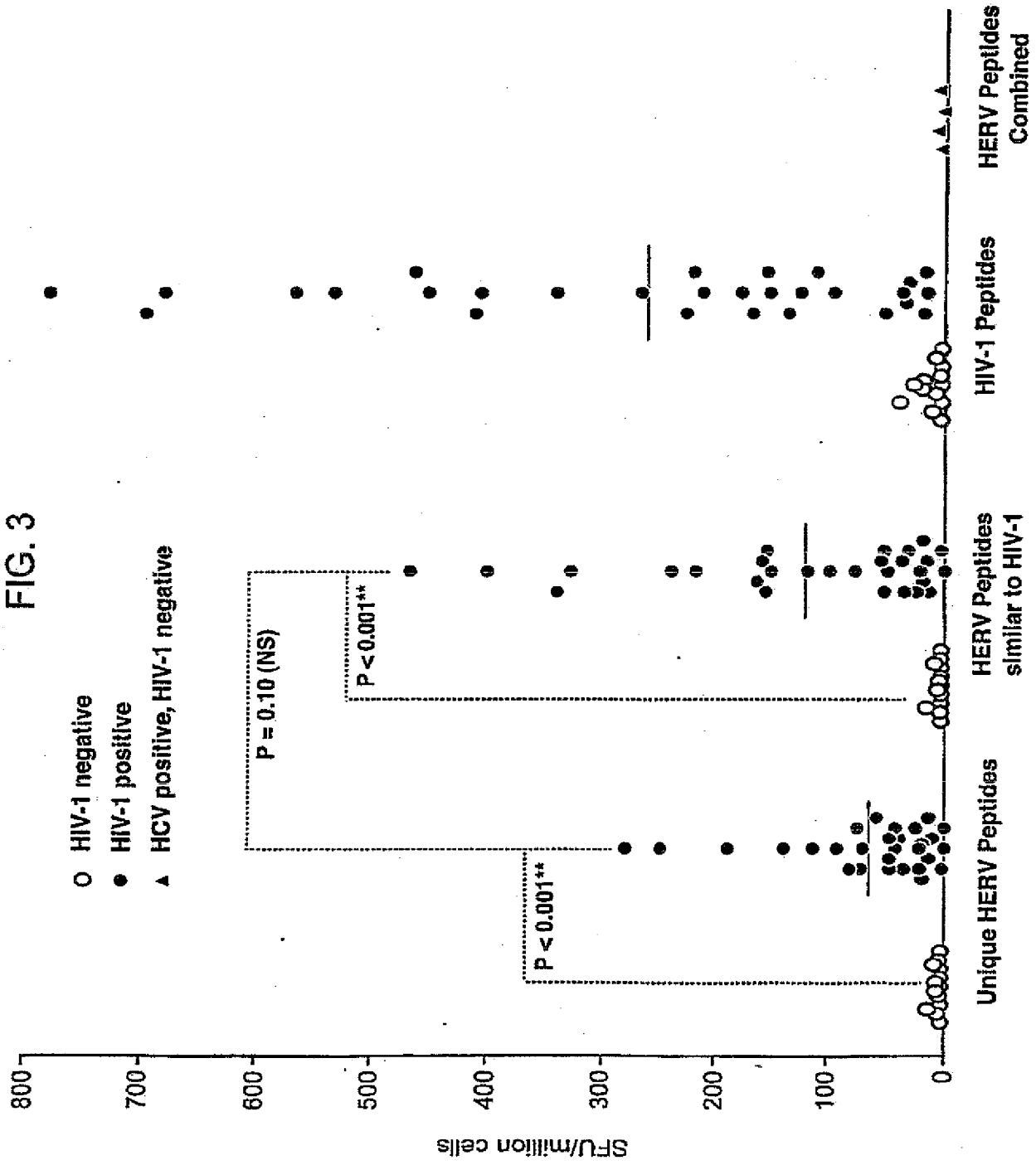

HIV-1 negative
HIV-1 positive
HCV positive, HIV-1 negative
P = 0.10 (NS)
P < 0.001**
P < 0.001**
SFU/million cells
800
700
600
500
400
300
200
100
0
Unique HERV Peptides
HERV Peptides similar to HIV-1
HIV-1 Peptides
HERV Peptides Combined

FIG. 4

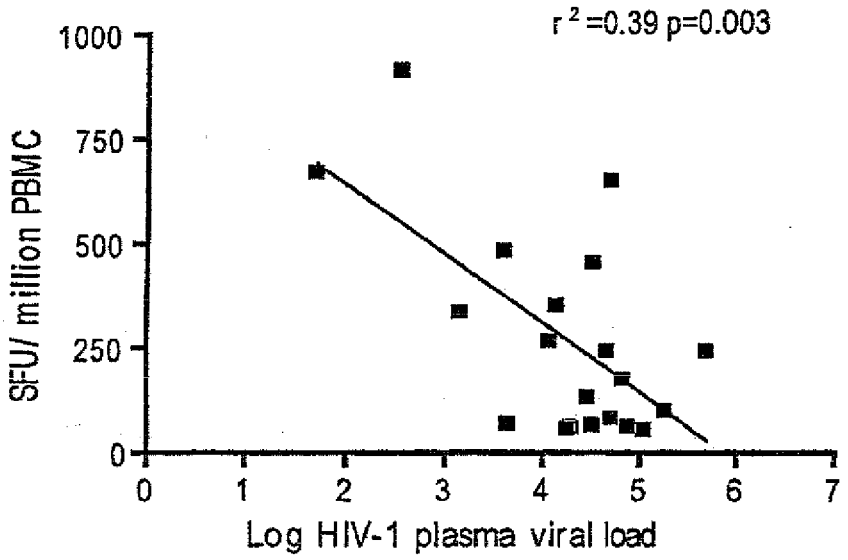
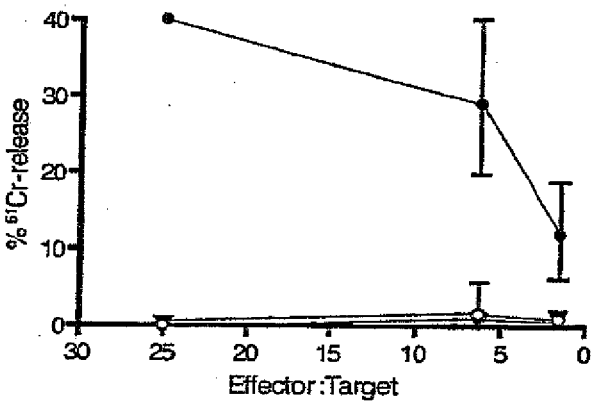

r 2 =0.39 p=0.003
1000
750
500
250
0
SFU/ million PBMC
0
1
2
3
4
5
6
7
Log HIV-1 plasma viral load

FIG. 5



—●— Targets pulsed with HERV-L IQ10 peptide.
—○— Targets pulsed with negative control (unrelated) peptide.
—▼— Unpulsed targets.

FIG. 6

HERV-K reverse transcriptase
GenBank AAD51797
gi:5802821

```
 990 s pwnspvfviq kksgkwrmlt dlravnaviq
1021 pmgplqpglp spamipkdwp liiidlkdcf ftiplaeqdc ekfaftipai nnkepatrfq
1081 wkvlpqgmln spticqtfvg ralqpvrekf sdcyiihcid dilcaaetkd klidcytflq
1141 aevanaglai asdkiqtstp fhylgmqi
(SEQ ID NO:31)
```

FIG. 7A

GenBank AJ233632
H. sapiens HERV-L pol gene; reverse transcriptase

RMIVDYRKLNKGSTPTAAAVSDVVSLLEQINTSPDTWYVATDLANAFCSIPVHKAHQKQFAFGWQGQEYTF
TVLSQGYINSPALCHNLVQRDLDHFSLPQDITLFHYID (SEQ ID NO:32)

FIG. 7B

H. sapiens HERV-L pol gene; reverse transcriptase

```
  1 agaatgatag tggattatcg taagcttaac aaagggtcta ctccaactgc agctgctgta
 61 tcagatgtag tttcattgct tgagcaaatt aacacatctc ctgatacctg gtatgtggcc
121 actgacttgg caaatgcctt ttgctccatt cctgtccata aggcccacca gaagcaattt
181 gcatttggct ggcaaggcca ggaatatacc ttcactgtcc tatctcaggg gtatatcaac
241 tctccagctt tgtgtcataa tcttgttcag agagatcttg atcacttttc acttccacaa
301 gatatcacac tattccatta cattgat (SEQ ID NO:33)
```

FIG. 8A

GenBank Accession No. Q9N2K0
Homo sapiens; HERV-H envelope

```
  1 mifagkapsn tstlmkfysl llysllfsfp flchplplps ylhhtinlth sllaasnpsl
 61 vnncwlcisl sssaytavpa vqtdwatspi slhlrtsfns phlyppeeli yfldrsskts
121 pdishqqaaa llrtylknls pyinstppif gplttqttip vaaplciswq rptgiplgnl
181 spsrcsftlh lrspttnine tigafqlhit dkpsintdkl knissnyclg rhlpcislhp
241 wlsspcssds pprpssclli pspennserl lvdtrrflih henrtfpstq lphqsplqpl
301 taaalagslg vwvqdtpfst pshlftlhlq fclaqglffl cgsstymclp anwtgtctlv
361 fltpkiqfan gteelpvplm tptqqkrvip liplmvglgl sastvalgtg iagistsvmt
421 frslsndfsa sitdisqtls vlqaqvdsla avvlqnrrgl dlltaekggl ciflneeccf
481 ylnqsglvyd nikklkdraq klanqasnya eppwalsnwm swvlpivspl ipiflllfg
541 pcifrlvsqf iqnriqaitn hsirqmfllt spqyhplpqd lpsa (SEQ ID NO:34)
```

FIG. 8B

GenBank Accession No. AF108843
Homo sapiens; HERV-H envelope

```
 642 atgatcttt gctggcaagg
 661 caccctccaa tacttccacc ctgatgaagt tctattcttt acttttatac tcactcttat
 721 tctcattccc attcttatgt catcctctac ctctccccag ctatctccac cacactatca
 781 accttaccca ttctctccta gccgcttcta atccctcctt agtgaacaac tgctggcttt
 841 gcatttccct ttcttccagt gcctacacag ctgtccccgc cttacagaca gactgggcaa
 901 catctcccat ctccctacac ctccgaactt cctttaacag ccctcacctt taccctcctg
 961 aagaactcat ttactttcta gacaggtcca gcaagacttc cccagacatt tcacatcagc
1021 aagctgccgc cctccttcgc acttatttaa aaaacctttc tccttatatc aactctactc
1081 ccccccatatt aggacctctc acaacacaaa ctactattcc tgtggccgct cctttgtgta
1141 tctcttggca aagacccact ggaattcccc taggtaatct ttcaccttct cgatgttcct
1201 ttactcttca tctccgaagt ccaactacaa acatcaatga aacaattgga gccttccagc
1261 tccatattac agacaagccc tctatcaata ctgacaaact taaaaacatt agcagtaatt
1321 attgcttagg aagacacttg ccctgtattt cactccatcc ttggctatct tccccttgct
1381 catcagactc tcctcccagg ccctcttctt gtttacttat acccagcccc gaaaataaca
1441 gtgaaagatt gctcgtagat actcgacgtt ttctcataca ccatgaaaat cgaaccttcc
1501 cctctacgca gttaccccat cagtccccat tacaacctct gacagctgcc gccctagctg
1561 gatccctagg agtctgggta caagacaccc ctttcagcac tccttctcac ctttttactt
1621 tacatctcca gttttgcctc gcacaaggtc tcttcttcct ctgtggatcc tctacctaca
1681 tgtgcctacc tgccaattgg acaggcacat gtacactagt cttccttacc cccaaaattc
1741 aatttgcaaa tgggaccgaa. gagctccctg ttcccctcat gacaccgaca caacaaaaaa
1801 gagttattcc actaattccc ttgatggtcg gtttaggact ttctgcctcc actgttgctc
1861 tcggtactgg aatagcaggc atttcaacgt ctgtcatgac cttccgtagc ctgtctaatg
1921 acttctctgc tagcatcaca gacatatcac aaactttatc agtcctccag gcccaagttg
1981 actctttagc tgcagttgtc ctccaaaacc gccgaggcct tgacttactc actgctgaaa
2041 aaggaggact ctgcatattc ttaaatgagg agtgttgttt ttacctaaat caatctggcc
2101 tggtgtatga caacattaaa aaactcaagg atagagccca aaaacttgcc aaccaagcaa
2161 gtaattacgc tgaaccccct tgggcactct ctaattggat gtcctgggtc ctcccaattg
2221 ttagtccttt aatacccatt tttctccttc ttttatttgg accttgtatc ttccgtttag
2281 tttctcaatt catccaaaac cgtatccagg ccatcaccaa tcattctata cgacaaatgt
2341 ttcttctaac atccccacaa tatcaccccc taccacaaga cctcccttca gcttaa
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(SEQ ID NO:36)