Abstract:
The disclosure encompasses \( p^{28}_{\text{TEV}} \) polypeptide, polynucleotides, variants, and antagonists. \( p^{28}_{\text{TEV}} \) polypeptides and/or polynucleotides are useful in immunogenic compositions. \( p^{15}_{\text{TEV}} \) polypeptide antagonists include antagonist antibodies, antisense molecules or siRNA molecules. The antagonists and composition of the disclosure can be administered alone or in combination with other agents useful in the treatment of HIV infection, SIV infection, AIDS, or AIDS-related complex (ARC), including nucleoside, non-nucleoside, and/or reverse transcriptase inhibitors.
ffIV TEV COMPOSITIONS AND METHODS OF USE

This application is being filed on 23 February 2007, as a PCT International Patent application in the name of the Government of the United States of America, as Represented by the Secretary, Department of Health and Human Services, applicant for all designated countries except the US, and Miroslav Dundr, Genoveffa Granchini, and Jake R. Fullen, applicants for the designation of the US only, and claims priority to U.S. Utility Patent Application No. 11/364,873, filed February 27, 2006.

Field of the Disclosure

This disclosure concerns human immunodeficiency virus (HIV) p28TEV protein and antagonists and methods of use, including methods of inhibiting HIV viral levels.

Statement of Rights to Disclosures Made Under Federally Sponsored Research And Development

The work performed during the development of this disclosure utilized intramural support from the National Institutes of Health. The United States Government has certain rights in the disclosure.

Background of the Disclosure

It is estimated that approximately 14,000 people worldwide are infected every day with human immunodeficiency virus (HTV). Many advances have been made in treating HIV infection and related Acquired Immune Deficiency Syndrome (AIDS), however, components of HIV that provide long lasting and effective immune responses remain to be identified. Unfortunately, HIV mutates rapidly resulting in multiple types and subtypes of the virus even in a single individual. Understanding how HIV infection operates in humans is an important component in developing effective therapies.

HIV type 1 (HIV-I) infects human CD4+ cells and persists despite a strong host immune response. The viral genome is reverse transcribed into a provirus that is integrated into the host genome. Butera, 2000, Antiviral Res., 48:143-176. In individuals undergoing highly active anti-retroviral therapy (HAART, regimens including various combinations of nucleoside, non-nucleoside, and protease inhibitors), levels of detectable virus remain for extended periods of time. Butera, 2000, Antiviral Res., 48: 143-176.

Tat and Rev are virally encoded regulatory factors for HIV gene expression. Tat acts by binding to the TAR RNA element and activating transcription initiation and/or elongation from the LTR promoter. Defects in the coding region of Tat or mutations that disrupt binding to TAR restrict HIV-I transcriptional activity. S.T. Butera, 2000, Antiviral...

HIV-I also encodes alternatively spliced mRNAs for several proteins involved in the regulation of transcription. In addition to Tat and Rev described above, two mRNAs encoding two proteins, p18 and p28\textsuperscript{TEV}, have also been described. Benko et al., 1990, J. Virol., 64:2505-25 18. HIV-I p28\textsuperscript{TEV} is encoded by alternatively spliced mRNA that joins together the first exon of Tat to a region with the Env (exon 6D) and to the second exon of Rev. The exon of the Env gene spliced with p28\textsuperscript{TEV} includes the V1 region of the virus. HIV-I p18 is produced by splicing exon 6D to the second exon of Rev. HIV-I p28\textsuperscript{TEV} exhibits both Tat and Rev function and can functionally replace both regulatory proteins in HIV-I. The function of the p28\textsuperscript{CV} and p18 in regulating viral expression in infected cells is not known. Human Retroviruses and AIDS 1996, Eds. Myers et al., Los Alamos National Laboratory, 1996 (accessible on the internet at http://hiv-web.lanl.gov).

A great deal of effort is being directed to the design and testing of agents that inhibit HIV infectivity or prevent HIV infection. Effective inhibitors of HIV infectivity are still needed.

**Summary**

One aspect of the disclosure is an immunogenic composition comprising at least one isolated nucleic acid encoding at least one p28\textsuperscript{TEV} polypeptide, preferably, a p28\textsuperscript{TEV} polypeptide having at least about 70 percent amino acid sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 151, SEQ ID NO: 143, SEQ ID NO: 146, SEQ ID NO: 1 and fragments thereof. In some embodiments the p28\textsuperscript{TEV} polypeptide retains transcriptional activation activity and/or the V1 region. In some embodiments, the composition may comprise one or more isolated nucleic acids encoding one or more p28\textsuperscript{TEV} polypeptides from one or more HIV-I clades. In some embodiments, the composition may comprise one or more isolated nucleic acids encoding one or more p28\textsuperscript{TEV} polypeptides selected from the group consisting of polypeptides comprising an amino acid sequence of SEQ ID NO:-143, SEQ ID NO:146, SEQ ID NO:1, and SEQ ID NO: 151 and fragments thereof. The fragments preferably include the V1 region. In other embodiments, the fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID
The composition may also comprise an immunomodulator, for example, an adjuvant. In some embodiments, the composition reduces or inhibits HIV viral levels upon administration to a mammal. Methods of using the immunogenic composition to immunize animals and reduce HIV viral levels are also provided.

Another aspect of the disclosure is a composition comprising a physiologically acceptable carrier and an effective amount of at least one isolated and/or purified p28\textsuperscript{TEV} polypeptide or an immunogenic fragment thereof. Preferably, the composition when administered reduces or inhibits viral levels. The composition may comprise p28\textsuperscript{TEV} polypeptides from one or more HIV-I clades. In some embodiments, the polypeptide comprises an amino acid sequence having at least about 70 percent and up to 100% sequence identity amino acid identity with a reference p28\textsuperscript{TEV}, including any number of sequence identity between 70% and 100% sequence identity. In some embodiments, the polypeptide has at least about 80 percent amino acid identity, has at least about 90 percent amino acid identity, or at least about 95% sequence identity with a reference p28\textsuperscript{TEV} polypeptide. In some embodiments, one or more of the p28\textsuperscript{TEV} polypeptides have the sequence of naturally occurring polypeptides from one or more HIV clades. In still another embodiment, the polypeptide is a fragment of a p28\textsuperscript{TEV} polypeptide comprising all or portion of the V1 region, preferably corresponding to amino acids 75-103 of p28\textsuperscript{TEV} of isolate 89.7. In still another embodiment, the reference polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ED NO: 143, SEQ ID NO: 146, SEQ ID NO: 151, and fragments thereof. In other embodiments, the referencing polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:137, SEQ ID NO: 138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:152, SEQ ID NO:153, SEQ IDNO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ED NO:160, SEQ ID NO:161, SEQ ID NO:162, SEQ ED NO:163, SEQ ID NO:164, and SEQ ID NO 165. The composition may also comprise an immunomodulator, for example, an adjuvant.

Another aspect of the disclosure is directed to methods of using an immunogenic composition to reduce or inhibit HTV viral levels. In some embodiments, a method comprises administering an immunogenic composition to a mammal comprising one or more isolated nucleic acids encoding a p28\textsuperscript{TEV} polypeptide. In some embodiments, the immunogenic composition comprises at least one isolated and/or purified p28\textsuperscript{TEV} polypeptide and, more preferably two or more p28\textsuperscript{TEV} polypeptides, each p28\textsuperscript{TEV} polypeptide from a different HIV clade. In some embodiments, the composition further comprises an immunomodulator or adjuvant. In some embodiments, the composition is administered to a human.
Another aspect is directed to an antibody that specifically binds a p28<sup>TEV</sup> polypeptide and an article of manufacture comprising the antibody. In some embodiments, the antibody inhibits or reduces HIV-1 levels. In some embodiments, the antibody inhibits p28<sup>TEV</sup> activity. In some embodiments, the polypeptide comprises an isolated p28<sup>TEV</sup> polypeptide comprising a sequence of SEQ ID NO: 151, SEQ ID NO:146, SEQ ID NO:1 or fragments thereof. The fragment preferably includes the V1 region. In other embodiments, the polypeptide comprises an amino acid sequence having at least about 70 percent and up to 100% sequence identity amino acid identity with a reference p28<sup>TEV</sup>, including any number of % sequence identity between 70% and 100% sequence identity. In some embodiments, the polypeptide has at least about 80 percent amino acid identity, has at least about 90 percent amino acid identity, or at least about 95% sequence identity with a reference p28<sup>TEV</sup> polypeptide. In some embodiments, one or more of the p28<sup>TEV</sup> polypeptides have the sequence of naturally occurring polypeptides from one or more HTV clades. In still another embodiment, the polypeptide or reference polypeptide is selected from the group consisting a polypeptide comprising the amino acid sequence of SEQ ID NO:1, SEQ IDNO:143, SEQ ED NO:146, SEQ ID NO:151, SEQ ID NO:137, SEQ ED NO:138, SEQ ED NO:139, SEQ ID NO:140, SEQ ID NO:152, SEQ ID NO:153, SEQ ED NO:154, SEQ ID NO:155, and SEQ ED NO:156. In some embodiments, an antibody that specifically binds a p28<sup>TEV</sup> polypeptide is generated by or binds to a consensus sequence of V1 from different clades comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 160, SEQ IDNO:161 3 SEQ EDNO:162, SEQ IDNO:163, SEQ ED NO:164, and SEQ ED NO:165. In some embodiments, the antibody specifically binds to the V1 region and does not specifically or significantly bind to the tat or rev region. The antibody may be a monoclonal antibody or a humanized antibody.

The article of manufacture comprises a container, an agent contained within the container, and a label, wherein the agent comprises at least one antibody or antigen binding fragment that binds specifically to a p28<sup>TEV</sup> polypeptide. The antibodies may be any combination of antibodies that specifically bind V1 in combination with anti-tat or anti-rev or both antibodies. The antibody or antigen binding fragment may be attached to a solid substrate. The solid substrate may be any suitable solid substrate, including the container or a multi-well plate. The antibody can be attached to a cytotoxic agent, siRNA molecule, or other inhibitor of HIV. The antibody or antigen binding fragment may be detectably labeled. The label may be a fluorescent moiety, radioactive moiety, peptide tag, or an enzyme.

The antibodies are also useful, for example, in methods for reducing viral levels or detecting virus in a host. In an embodiment, the method comprises a) contacting a
biological sample with at least one antibody that specifically binds a p28\textsuperscript{TEV} polypeptide and
b) detecting the presence of p28\textsuperscript{TEV} in the biological sample.

Another aspect of the disclosure is an antagonist of p28\textsuperscript{TEV} comprising an isolated polynucleotide that inhibits expression of p28\textsuperscript{TEV}, vectors comprising the antagonist, and

host cells comprising the vector. The polynucleotide may inhibit expression directly or encode a molecule that inhibits expression of p28\textsuperscript{TEV}. In an embodiment, the polynucleotide is an antisense RNA molecule. In another embodiment, the polynucleotide encodes an antisense RNA molecule. In another embodiment, the polynucleotide is a small interfering RNA (siRNA) molecule. In still another embodiment, the polynucleotide encodes a siRNA molecule.

In some embodiments, the polynucleotide hybridizes under stringent conditions to a nucleic acid molecule encoding p28\textsuperscript{TCV} or a p28\textsuperscript{TEV} splice acceptor site and/or a p28\textsuperscript{TEV} splice donor site. In an embodiment, the polynucleotide comprises a polynucleotide sequence of SEQ ID NO:2. The stringent conditions may be stringent, moderately stringent, or highly stringent. In an embodiment, the nucleic acid molecule encoding p28\textsuperscript{TEV} comprises a polynucleotide sequence encoding an ENV exon. In a preferred embodiment, the exon is exon 6D. In an embodiment, the nucleic acid molecule encoding a p28\textsuperscript{TEV} splice acceptor site comprises a polynucleotide sequence of SEQ ID NO:3. In another embodiment, the nucleic acid molecule encoding the p28\textsuperscript{TEV} splice acceptor site comprises a splice acceptor site at nucleotide 6154 of mRNA of HXB2. In an embodiment, the nucleic acid molecule encoding a p28\textsuperscript{TEV} splice donor site comprises a polynucleotide sequence of SEQ ID NO:4. In another embodiment, the nucleic acid molecule encoding the p28\textsuperscript{TEV} splice donor site comprises a splice acceptor site at nucleotide 6269 of mRNA of HXB2.

Antagonists comprising a polynucleotide that inhibits expression of p28\textsuperscript{TEV} are useful, for example, in methods for reducing viral levels. In an embodiment, the method comprises administering to a patient in need thereof an effective amount of the antagonist to inhibit HIV-I viral levels.

The present disclosure is also directed to methods for identifying antagonists of p28\textsuperscript{TEV} polypeptide. In an embodiment, the method comprising contacting a p28\textsuperscript{TEV} polypeptide or cell comprising a polynucleotide encoding a p28\textsuperscript{TEV} polypeptide with a candidate agent, and determining whether the candidate agent inhibits an activity or expression of p28\textsuperscript{TEV} polypeptide. A candidate agent that inhibits the activity or expression of p28\textsuperscript{TEV} polypeptide is identified as an antagonist of the p28\textsuperscript{TEV} polypeptide. The antagonist may reduce HIV viral levels and/or transcriptional activity of p28\textsuperscript{TEV}. Candidate agents include aptamers, anti-p28\textsuperscript{TEV} antibodies, antisense, and siRNA.

Another aspect of the disclosure is a method for inhibiting HIV viral levels. In an embodiment, the method comprises administering to a patient in need thereof an effective
amount of an antagonist. In some embodiments, the antagonist is administered as a therapeutic agent to a patient infected with HIV, either alone or in combination with other antiviral therapies. In a preferred embodiment, the antagonist is an antibody. The antibody may be monoclonal. In an embodiment, the monoclonal antibody is humanized. In another embodiment, the monoclonal antibody is a Fv, Fab, Fab', or F(ab')2 fragment. In another preferred embodiment, the antagonist is a polynucleotide as described above.

Another aspect of the disclosure is a method for inhibiting or reducing HIV viral levels. In an embodiment, the method comprises administering an effective amount of an immunogenic composition to a mammal. In some embodiments, the immunogenic composition comprises one or more isolated nucleic acids encoding p28TEV, optionally in combination with an immunomodulator. In other embodiments, the immunogenic composition comprises one or more isolated and purified p28TEV polypeptides, preferably from different HIV clades, optionally in combination with an immunomodulator. In some embodiments, the immunogenic composition comprises one or V1 peptides optionally in combination with an immunomodulator.

The antagonists or immunogenic compositions of the disclosure can be administered in combination with other agents useful in the treatment of HIV infection, SIV infection, AIDS, or AIDS-related complex (ARC), including nucleoside, non-nucleoside, and/or reverse transcriptase inhibitors.

Brief Description of the Figures

Figure 1 shows that p28TEV is expressed in the nucleolus and cytoplasm of HeLa cells transfected with a vector encoding p28TEV fused to GFP at the C terminus. HTLV-I p30H1 protein fused at the C terminus to GFP was used as a control and is expressed in the nucleolus only.

Figure 2A shows the efficiency of transfection of 293T cells in each experimental condition using the RLTK construct. Cells were co-transfected with vectors encoding pHXB2 (Fisher et al., 1986, Science, 233:655-659); p28TEV; RL-TK (Promega Corp., Madison, WI); and HIV-Luc (M. R. Smith and W. C. Greene, 1990, Genes Dev., 4:1875-1885). The cells were transfected with increasing amounts of the vector encoding HXB2 p28TEV from 0.5 to 10 ug. Transfection efficiency was determined by measuring expression levels of Renilla luciferase. The results show equivalent RLTK expression in all the experimental conditions.

Figure 2B shows that increasing amounts of HXB2 p28TEV expression increased luciferase from the HTV-Luc-reporter gene. The HIV-Luc reporter gene was constructed using the 5' LTR region of HIV-I strain fused to the firefly luciferase reporter gene. p28TEV
contains the Tat-activating domain. The results show stimulation of transcription activity of HIV-I LTR in the presence of increasing amounts of trans HXB2 p28^{TEV}.

Figure 2C shows that even though the Tat activity increased in the presence of increasing amounts of trans p28^{TEV}, viral production as measured by p24 in the supernatant decreased.

Figure 2D shows a western blot of HIV-I p24. The results show that intracellular expression of HW-I p24 was decreased with increasing amounts of expression of p28^{TEV} whereas the level of α-tubulin, a housekeeping protein, remained constant. p28^{TEV} was also detected in the supernatant of the transfected cells. Increasing amounts of p28^{TEV} were added to lanes starting from left to right.

Figures 3A shows the increase in expression of HIV-Luc in the presence of increasing amounts of trans HXB2 p28^{TEV} and no HIV-Luc activity in the absence of the HIV-Luc reporter gene (last three lanes).

Figure 3B shows a dose-dependent decrease of p24 in the supernatant in the presence of increasing amounts of HXB2 p28^{TEV} both in the presence and absence of HIV-Luc indicating that exogenous HIV-1-Luc is not competing for the transactivation of Tat produced by the provirus.

Figure 4 shows a representative amino acid sequence of HIV-I p28^{TEV} (SEQ ID NO:1) from HXB2.

Figure 5 shows a representative nucleic acid sequence of HIV-I p28^{TEV} (SEQ ID NO:2) from HXB2.

Figure 6 shows the position of the splice acceptor site and splice donor site in a nucleic acid construct encoding p28^{TEV}.

Figure 7 shows an alignment of DNA sequences of representative HIV-1 isolates wherein the reference sequence is HXB2. Other similar sequences can be found at the Los Alamos database. The location of the splice acceptor site for p28^{TEV} in mRNA of HXB2 is identified. (SEQ ID NOs: 14-74)

Figure 8 shows an alignment of DNA sequences of representative HIV-1 isolates wherein the reference sequence is HXB2. Other similar sequences can be found at the Los Alamos database. The location of the splice donor site for p28^{TEV} in mRNA of HXB2 is identified. (SEQ ID NOs: 75-135)

Figure 9. Top: schematic representation of the 3′ ORF that participates in the generation of Tev. Middle: DNA sequence of the primer used to mutagenize the 6 nucleotides within the acceptor splice site for Tev in the pHXB2, pNL4, and 89.6 mutant clones (bottom). The sequence of the primer is the following:

5'-CTCTGTGTTTCACTGAAGTGCACT-3' (SEQ ID NO:1)
Figure 10 293T cells were transfected with 10 µg each of pHXB2 or pHXB2ΔTev. p24 levels were measured on the supernatant at 24 and 48 h (top panel). Western blots of the cell lysates are shown in the bottom panels. Rev expression was augmented in ΔTev clones. The expression and cleavage of the Env proteins was not affected. This result was expected as the Env precursor is cleaved by cellular protease.

Figure 11: Panel A: 293T cell cultures were separately transfected with 10 µg each of pME, pHXB2, pHXB2ΔTev, pNL, pNL4-3ΔTev, 89.6, and 89.6ΔTev. p24 levels were measured on the supernatant at 48 h.

Panel B: Western blot of cell lysates using antibodies to gpl60 and CD71.

Panel C: 293T cells were separately transfected with 10 µg each of pME, pNL4-3, and increasing amounts of cDNA encoding Rev and p24 production was measured.

Panel D: TZM cells were exposed to equivalent amounts of p24 from the supernatant of cells transfected with pME, pHXB2, pHXB2ΔTev, pNL, pNL4-3ΔTev, 89.6, and 89.6ΔTev. The cells were lysed at 48 h and luciferase activity measured.

Panel E: TZM cell cultures exposed separately to the supernatant from pME, pHXB2, pHXB2ΔTev, pNL, pNL4-3ΔTev, 89.6, and 89.6ΔTev. Transfected cells were lysed at 48 h and reverse transcriptase activity measured.

Figure 12: (A) Amino acid alignment (in single letter code) of HIV HXB2, BaL, SF162, and 89.6P Tev proteins (SEQ ID NOS: 1, 143, 146, 151). (B) Expression of cDNAs encoding recombinant Tev proteins from BaL, pHXB2, SFI 62, and 89.6P HIV strains in 293T cells. (C) Electrophoretic analysis of the purified 89.6P protein expressed in bacteria as detected by Coomassie blue staining (Lane 1) and molecular weight markers (Lane 2). (D) Schematic representation of the study design and immunization regimen of macaques.

Animals in group 1 received three immunizations with a mixture of 4 mg each of a cDNAs encoding HXB2, BaL, SF162, and 89.6P Tev proteins by the intramuscular route and boosted with 200 µg of 89.6P Tev protein together with 1 mg of CpG class B at the time indicated. Intravenous challenge with highly pathogenic chimeric SHTV89.6P was performed at week 22.

Figure 13A shows viral loads of animals immunized with a representative immunogenic composition. Figure 13B shows viral loads of control animals that were not immunized.

Figure 14A shows CD3+/CD4+ T-cell counts of immunized animals. Figure 14B shows CD3+CD4+ T-cell counts of control animals.

Figure 15 shows ELISPOT responses in control and vaccinated animals to (A) BaL, (B) SF162, and (C) 89.6 Tev in animals after challenge with SHIV89.6P was
performed at week 22 (day of challenge) and thereafter; Panel (D) shows the response of T cells to Con A as a control of cell viability.

Figure 16 shows antibody titers to HIV-I BOB Tat in animals immunized with a representative immunogenic composition and control animals before and after challenge with SHIV89.6P.

Figure 17 shows antibody titers to HIV-I MN Rev in animals immunized with a representative immunogenic composition and control animals before and after challenge with SHIV89.6P.

Figure 18 shows antibody titers to HIV-I Env V1 in animals immunized with a representative immunogenic composition and control animals before and after challenge with SHIV89.6P. The peptides used in this assay were

- BAL: CRNATNGNDTNSSSR (SEQ ID NO: 157)
- SF 162: KNATNTKSSNWKEMDCR (SEQ ID NO: 158)
- 89.6: CKNTNTPTSSSWGMMEK (SEQ ID NO: 159)

Figure 19 ELISA Ab response to overlapping peptides from 89.6P Tev proteins on immunized animals 316 and 308 at week 10, week 19, week 24 (post challenge with SHIV89.6 at week 22), and week 27. An increase in antibodies to peptides 21-24 of Tev corresponding to V1 is seen and is outlined.

Figure 20 ELISA Ab response to overlapping peptides from 89.6P Tev proteins on immunized animals 490 and 218 at week 10, week 19, week 24 (post challenge with SHIV89.6 at week 22), and week 27. An increase in antibodies to peptides 21-24 of Tev corresponding to V1 is seen in 490 but not in 218 and is outlined.

Figure 21 ELISA Ab response to overlapping peptides from 89.6P Tev proteins on immunized animal 316 at week 10, week 19, week 24 (post challenge with SHIV89.6 at week 22), and week 27. An increase in antibodies to peptides 21-24 after corresponding to V1 is seen even at 1:5,000 serum and is outlined.

Figure 22 ELISA Ab response to overlapping peptides from 89.6P Tev proteins on control animals 915 and 320 at week 10, week 19, week 24 (post challenge with SHTV89.6 at week 22), and week 27. No increase in antibodies to peptides 21-24 after corresponding to V1 is seen.

Figure 23 (A) ELISA Ab response to overlapping peptides from 89.6P Tev proteins on immunized animals 316, 308, 218, 490 and control animals 915 and 320 at week 10, week 19, week 24 (post challenge with SHIV89.6 at week 22), and week 27. An increase in antibodies to peptides 21-24 of 89.6 Tev corresponding to V1 is seen post challenge in the immunized animals; (B) ELISA response to overlapping peptides from BaL Tev proteins on immunized animals 316, 308, 218, 490 and control animals 915 and 320 at week 10, week 19, week 24 (post challenge with SHTV89.6 at week 22), and week 27. No cross-reactivity
of antibodies to peptides 21-24 corresponding to BaL Tev V1 is seen; (C) ELISA response to overlapping peptides from SF162 Tev proteins on immunized animals 316, 308, 218, 490 and control animals 915 and 320 at week 10, week 19, week 24 (post challenge with SHIV89.6 at week 22), and week 27. No cross-reactivity of antibodies to peptides 21-24 corresponding to SF162 Tev V1 is seen.

Figure 24 shows the SIV RNA load in different tissues: lymph nodes, jejunum, spleen, and plasma, at the time of sacrifice (6 months from challenge exposure). Animals M320 and 915L are control nonimmunized animals.

Figure 25 (A) shows the results of antibody dependent cellular viral inhibition (ADCVI) of plasma samples (diluted 1:100) from immunized and nonimmunized animals at week 22, week 26 and week 28. All animals were challenged at week 22 with SHIV89.6. Animals 915 and 320 are nonimmunized control animals. (B) antibody dependent cell mediated virus inhibition of serial dilutions of plasma samples from immunized and nonimmunized animals at 26 weeks. (C) ADCVI of serial dilutions of plasma samples from immunized and nonimmunized animals at 28 weeks.

Figure 26 shows the polynucleotide sequence encoding tev from the BaL HIV isolate. (SEQ ID NO: 141) This sequence is codon optimized for expression in humans and E. coli. The lower sequence is a synthetic gene sequence prepared as described in the examples, sequenced and compared to the sequences provided (top sequence).

Figure 27 shows the polynucleotide (SEQ ID NO: 142) and amino acid sequence for Tev (SEQ ID NO: 143) from the BaL HIV isolate from plasmid pPCR-Script Amp.

Figure 28 shows the polynucleotide sequence encoding tev from the SF162 HTV isolate. (SEQ ID NO: 144) This sequence is codon optimized for expression in humans and E. coli. The lower sequence is a synthetic gene sequence prepared as described in the examples, sequenced and compared to the sequences provided (top sequence).

Figure 29 shows the polynucleotide (SEQ ID NO: 145) and amino acid sequence for tev (SEQ ID NO:146) from the SF162 HIV isolate from plasmid pPCR-Script Amp.

Figure 30 shows a polynucleotide sequence encoding tev from the HXB2 HIV isolate. (SEQ ID NO: 147) This sequence is codon optimized for expression in humans and E. coli. The lower sequence is a synthetic gene sequence prepared as described in the examples, sequenced and compared to the sequences provided (top sequence).

Figure 31 shows a polynucleotide (SEQ ID NO: 148) and amino acid sequence for tev (SEQ ID NO: 1) from the HXB2 HIV isolate from plasmid pPCR-Script Amp.

Figure 32 shows a polynucleotide sequence encoding tev from the 89.6 HIV isolate.
Figure 33 shows the polynucleotide (SEQ ID NO: 150) and amino acid sequence (SEQ ID NO: 151) for tev from the 89.6 HIV isolate from plasmid pPCR-Script Amp.

Figure 34 shows the plasmid maps for each plasmid that has a polynucleotide encoding tev. Panel A is the plasmid map for BaL tev. Panel B is the plasmid map for SF162 tev. Panel C is the plasmid map for HXB2 tev. Panel D is the plasmid map for 89.6 tev.

Figures 35A and B show an alignment of V1 sequences from several different HIV isolates or strains obtained from the Los Alamos database. The consensus sequences for the V1 region of each clade can be identified by comparing the sequences between the two cysteines identified by the first two arrows. (SEQ ID NOS: 166-299)

**Detailed Description**

**I. Definitions**

The terms "p28^{TEV} polypeptide" or "p28^{TEV} protein" or "p28^{TEV}" or "p28" are used interchangeably and encompass both naturally occurring p28^{TEV} polypeptides or proteins and p28^{TEV} polypeptide variants. In an embodiment, p28^{TEV} polypeptide comprises an amino acid sequence of SEQ ID NO: 151 (Figure 33). In other embodiments, p28^{TEV} polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ED NO: 1, SEQ ED NO: 143, SEQ ID NO: 146, and SEQ ID NO: 151.

The terms "naturally occurring p28^{TEV} polypeptide" or "naturally occurring p28^{TEV}" are used interchangeably and encompass polypeptides that have the same amino acid sequence of a polypeptide obtained from nature from HIV or an HIV infected cell. The terms "naturally occurring p28^{TBV} polypeptide" or "naturally occurring p28^{TBV}" specifically encompasses any of the naturally occurring forms of the polypeptides including polypeptides with a signal sequence and/or mature forms without the signal sequence. Naturally occurring variants include secreted forms, alternatively spliced forms, and those naturally occurring variants from other HTV-I strains or isolates that differ in sequence from a reference sequence for a particular p28^{TEV} polypeptide. In an embodiment, the reference sequence comprises an amino acid sequence of SEQ ID NO: 151. In some embodiments, when the reference sequence p28^{TEV} is SEQ ID NO: 1, then other naturally occurring variants have at least 80% sequence identity to SEQ ID NO:1. See, for example, Figure 12A.) Naturally occurring p28^{TEV} polypeptides are expressed early in infection and have a biological activity of transcriptional activation. Naturally occurring p28^{TEV} polypeptides or proteins can be isolated or purified from nature, prepared recombinantly or synthetically.

"p28^{TEV} polypeptide variant" or "p28^{TEV} protein variant" or "p28^{TEV} variant" refers to a p28^{TEV} polypeptide that differs in amino acid sequence from a particular p28^{TEV} polypeptide reference sequence. In an embodiment, the p28^{TEV} polypeptide reference
sequence comprises an amino acid sequence of SEQ ID NO: 151. "p28TEV variant polypeptides" or "p28TEV variant proteins" or "p28TCV variants" specifically encompasses modifications of the reference sequence, and naturally occurring p28TEV polypeptide variants. When the variant is a naturally occurring p28TEV polypeptide variant of the reference sequence, the variant is designated "a naturally occurring p28TEV variant." The variants may include deletions and additions of amino acids, as well as amino acid substitutions.

A p28TEV variant has at least about any number of % sequence identity from 70% to 100 % sequence identity to a full-length mature p28TEV polypeptide reference sequence. A p28TEV variant has at least about 70% sequence identity, more preferably at least about 75% sequence identity, more preferably at least about 80% sequence identity, more preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, more preferably at least about 95% sequence identity and even 100% sequence identity to a full-length mature p28TEV polypeptide reference sequence, such as a polypeptide having the sequence of SEQ ID NO: 151 or a polypeptide fragment comprising amino acids 73-12 of SEQ ID NO: 151. In some embodiments, a p28TEV variant has at least 70% sequence identity in the VI region as shown, for example, in Figure 12A. In other embodiments, a p28TEV reference polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 146, SEQ H>NO: 151, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, and SEQ ID NO: 156. In some embodiments, the polypeptide variants increase Tat transcriptional activity in the HIV-1-Luc assay at least 2 fold.

The term "isolated," when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Preferably, the isolated polypeptide is free of association with at least one component with which it is naturally associated. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide and may include enzymes, and other proteinaceous or non-proteinaceous solutes. An isolated polypeptide includes polypeptide in situ within recombinant cells, since at least one component of the p28TEV polypeptide natural environment will not be present. Ordinarily, however, an isolated polypeptide will be prepared by at least one purification step.

An "isolated" nucleic acid molecule encoding a p28TEV polypeptide is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the p28TEV-encoding nucleic acid. Preferably, the isolated nucleic is free of association with all components with
which it is naturally associated. An isolated p28\textsuperscript{TEV}-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the p28\textsuperscript{TEV}-encoding nucleic acid molecule as it exists in natural cells or virus. In an embodiment, the nucleic acid molecule comprises a nucleic acid sequence of SEQ ID NO:2. In other embodiment, the nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:144, SEQ ED NO:145, SEQ ID NO:147, SEQ do NO:148, SEQ IDNO:149, and SEQ ID NO:150. In another embodiment, the nucleic acid molecule encodes ap28 \textsuperscript{TEV} polypeptide comprising the amino acid sequence of SEQ ID NO:151 or variants thereof. In other embodiments, the nucleic acid molecule encodes a p28\textsuperscript{TEV} polypeptide that comprises an amino acid sequence selected from the group consisting of SEQ DDNO:1, SEQ DDNO:143, SEQ DDNO:146, SEQDDNO:151, SEQ ID NO:136, SEQIDNO:137, SEQ DDNO:138, SEQ DDNO:139, SEQDDNO:140, SEQ ID NO:152, SEQ IDNO:153, SEQ IDNO:154, SEQ IDNO:155, SEQ IDNO:156, SEQ HO NO:160, SEQIDNO:161, SEQ IDNO:162, SEQ vNO:163, SEQ DDNO:164, and SEQ ID NO:165.

The disclosure also includes variants of nucleic acid molecules encoding p28\textsuperscript{TEV} polypeptides. In one embodiment, the disclosure includes polynucleotides encoding a polypeptide having at least about any number of sequence identity from 70% to 100% sequence identity to the reference polypeptide for p28\textsuperscript{TEV}, more preferably about 70% sequence identity, more preferably about 75% sequence identity, more preferably about 80% sequence identity, more preferably about 85% sequence identity, more preferably about 90% sequence identity, more preferably about 95% sequence identity, and even up to 100% sequence identity to a reference p28\textsuperscript{TEV} protein such as that having an amino acid sequence of SEQ ID NO:151. In other embodiments, a p28\textsuperscript{TEV} reference polypeptide comprises an amino acid sequence selected from the group consisting of SEQ do NO:1, SEQ ID NO:143, SEQ ID NO:146, SEQ ID NO:151, SEQ ID NO:137, SEQ do NO:138, SEQ do NO:139, SEQ do NO:140, SEQ do NO:152, SEQ do NO:153, SEQ ID NO:154, SEQ do NO:155, and SEQ do NO:156. In some embodiments, the polynucleotide variants encode a polypeptide that increases Tat transcriptional activity in the HIV-1-Luc assay.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable include, for example, a promoter, and optionally an enhancer sequence.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to
a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

Peptide and protein sequences defined herein are represented by one-letter symbols for amino acid residues as follows:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Ala alanine</td>
</tr>
<tr>
<td>R</td>
<td>Arg arginine</td>
</tr>
<tr>
<td>N</td>
<td>Asn asparagine</td>
</tr>
<tr>
<td>D</td>
<td>Asp aspartic acid</td>
</tr>
<tr>
<td>C</td>
<td>Cys cysteine</td>
</tr>
<tr>
<td>Q</td>
<td>Gln glutamine</td>
</tr>
<tr>
<td>E</td>
<td>Glu glutamic acid</td>
</tr>
<tr>
<td>G</td>
<td>Gly glycine</td>
</tr>
<tr>
<td>H</td>
<td>His histidine</td>
</tr>
<tr>
<td>I</td>
<td>Ile isoleucine</td>
</tr>
<tr>
<td>L</td>
<td>Leu leucine</td>
</tr>
<tr>
<td>K</td>
<td>Lys lysine</td>
</tr>
<tr>
<td>M</td>
<td>Met methionine</td>
</tr>
<tr>
<td>F</td>
<td>Phe phenylalanine</td>
</tr>
<tr>
<td>P</td>
<td>Pro proline</td>
</tr>
<tr>
<td>S</td>
<td>Ser serine</td>
</tr>
<tr>
<td>T</td>
<td>Thr threonine</td>
</tr>
<tr>
<td>W</td>
<td>Trp tryptophan</td>
</tr>
<tr>
<td>Y</td>
<td>Tyr tyrosine</td>
</tr>
<tr>
<td>V</td>
<td>Val valine</td>
</tr>
</tbody>
</table>

"Percent (%) amino acid sequence identity" with respect to the p28 \textsuperscript{TEV} polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a p28 \textsuperscript{TEV} polypeptide reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, clustal V (DNASTAR) or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. Alignments of some HIV proteins from different clades can be found at the Los Alamos website (http://www-hiv-lanl.gov/content/index).

For purposes herein, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid
sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

\[ 100 \times \text{the fraction } X/Y \]

where X is the number of amino acid residues scored as identical matches by the sequence alignment program in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Typically, the B amino acid sequence is that of SEQ ID NO: 1.

"Percent (%) nucleic acid sequence identity" with respect to the p28\textsuperscript{TEV} polypeptide-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in a reference p28\textsuperscript{TEV} polypeptide-encoding nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, or Megalign (DNASTAR) software. Alignments of some HIV nucleic acids from different clades can be found at the Los Alamos website (http://www-hiv-lanl.gov/content/index). Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared.

For purposes herein, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

\[ 100 \times \text{the fraction } W/Z \]

where W is the number of nucleotides scored as identical matches by the sequence alignment program in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C.

The term "antibody" is used in the broadest sense and specifically includes, for example, single anti-p28\textsuperscript{TEV} monoclonal antibodies, anti-p28\textsuperscript{TEV} antibody compositions with polyepitopic specificity, human antibodies, humanized antibodies, single chain anti-p28\textsuperscript{TEV} antibodies, and fragments of anti-p28\textsuperscript{TEV} antibodies (see below). The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially
homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a p28TEV polypeptide linked to a "peptide tag". The peptide tag has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The peptide tag preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable peptide tags generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

As used herein "fusion protein" refers to an additional polypeptide that is linked to a p28TEV polypeptide, preferably, at the N and/or C terminal end. The additional polypeptide is preferably a peptide tag that provides for ease of purification or identification. Additional peptides or polypeptides may also be fused to enhance immunogenicity, such as bovine serum albumin, or keyhole lymphocyte hemocyanin.

An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. Isolated antibody includes the antibody *in situ* within
recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The term "immunogenic effective amount" of a p28TCV polypeptide or polynucleotide disclosed herein refers to an amount of a polypeptide that is capable of inducing an immune response in an animal. The immune response may be determined by measuring a T or B cell response. Typically, the induction of an immune response is determined by the detection of antibodies specific for a p28TEV polypeptide.

As used herein, the term "immunogenic fragment thereof" refers to a fragment a p28TEV polypeptide that is of a sufficient size to elicit an immune response in an animal. Typically, immunogenic fragments are at least 8 amino acids long and may include up to the full-length polypeptide. In some embodiments, an immunogenic fragment is about 10 amino acids, 15 amino acids, 30 amino acids, or 45 amino acids. The immunogenic fragment is capable of stimulating an antibody or selecting an antibody that specifically binds to at least one p28TEV polypeptide. In some embodiments, the immunogenic fragment includes overlapping polypeptides in the V1 region of a p28TEV polypeptide. The immune response includes both a T and B cell response, but preferably is identified by the ability of the fragment to elicit antibodies.

The term "binds specifically" refers to an antibody that binds to a particular p28TEV polypeptide. In some embodiments, the antibodies are specific for peptides of the V1 region. In some embodiments, the antibody specifically binds p28TEV and does not bind to Tat or Rev. In other embodiments, the antibody that binds to p28TEV may crossreact with Tat and/or Rev, but binds to p28TEV with a higher affinity, preferably at least 100 fold higher affinity, more preferably 1000 fold higher affinity or greater than binding of the antibody to Tat and/or Rev in order to allow for differential detection of p28TEV from tat or rev proteins.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures.

Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley InterScience Publishers, (1995).
"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SW (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and % SDS) less stringent that those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, in etc. as necessary to accommodate factors such as probe length and the like.

As used herein "recombinant" refers to a nucleic acid molecule that has been isolated and/or altered by the hand of man. Typically a DNA sequence encoding a polypeptide is isolated and combined with other control sequences in a vector. The other control sequences may be those that are found in the naturally occurring gene or others. The vector provides for introduction into host cells, amplification of the nucleic acid sequence and expression of the nucleic acid sequence.

As used herein, the term "treating" refers to therapeutic treatment. Those in need of treatment include those already infected with HIV, especially those having a reservoir of virus.

"siRNA" refers to a small interfering RNA that is a short-length double-stranded RNA and is not toxic in mammalian cells. siRNA induces gene-specific suppression through sequence specific degradation of homologous gene transcripts. P. Sharp, 1999, Genes & Devlop., 13:139-141; Berstein et al., 2000, RNA, 97:4985; U.S. 20030220287. There is no particular limitation in the length of siRNA. U.S. 20040002077. The siRNA may be 15 to 50 bp long, preferably 15 to 35 bp, and more preferably 21 to 30 bp. The double-stranded
RNA portions of siRNAs may contain nonpairing nucleotides due to mismatches, wherein the corresponding nucleotides are not complementary, and/or bulges, wherein the corresponding complementary nucleotide is lacking on one strand. See U.S. 2004/0002077. Double stranded RNA (dsRNA) may comprise nonpairing nucleotides to the extent the nonpairing nucleotides do not interfere with siRNA formation.

"Antisense RNA" refers to a single strand RNA comprising a sequence that is complementary to a target mRNA and not toxic in mammalian cells. There is no particular limitation in the length of antisense RNA. See U.S. 2003/0220287. The length of antisense RNA is dependent upon the number and composition of complementary bases and the accessibility of the target sequence. U.S. 2003/0220287.

A. Immunogenic Compositions

An embodiment of the present disclosure provides an immunogenic composition or pharmaceutical composition including at least one isolated and/or purified p28<sup>TEV</sup> polypeptide, an isolated nucleic acid encoding at least one p28<sup>TEV</sup> polypeptide, or both. In some embodiments, the immunogenic composition comprises an isolated naturally occurring p28<sup>TEV</sup> polypeptide or isolated nucleic acid encoding a naturally occurring p28<sup>TEV</sup> polypeptide, or both, from more than one clade of HIV. Preferably, an immunogenic composition comprises at least two and up to six different polynucleotides and/or polypeptides. A single nucleic acid may encode more than one p28<sup>TEV</sup> polypeptide and/or a nucleic acid may encode a single p28<sup>TEV</sup> polypeptide and/or combinations thereof. In some embodiments, compositions contain an amount of p28<sup>TEV</sup> polypeptide and/or polynucleotide effective to elicit an immune response when administered to a host, for example, a mammal. The immune response can be humoral, cellular, or a both. Generally, the immune response inhibits the HIV viral levels in the immunized host compared to HIV viral levels in non-immunized hosts. The immunogenic composition optionally includes a pharmaceutically acceptable excipient or carrier.

An embodiment provides an immunogenic composition according to the present disclosure also including immunomodulators such as cytokines or chemokines. In some embodiments, a nucleic acid encodes the immunomodulator or adjuvant. Immunomodulators refers to substances that potentiate an immunogenic response including, but not limited to cytokines and chemokines. Examples of cytokines include but are not limited to IL-2, EL-15, EL-12, or GM-CSF. Adjuvants such as lipids (fatty acids, phospholipids, Freund's incomplete adjuvant in particular), anionic copolymers, CpG units, etc. may be added to the composition.

In certain embodiments, the immunogenic composition comprises at least one isolated nucleic acid encoding a p28<sup>TEV</sup> polypeptides or fragments thereof, recombinant vector or DNA. Recombinant vector refers to vectors that replicate in a host and express an
antigen such as p28\textsuperscript{TEV}. One example of such a vector is a poxvirus. Poxviruses (including canarypox, vaccinia, and fowlpox) are suitable for recombinant vectors comprising an immunogenic composition disclosed herein. Poxviruses are capable of accommodating large amounts of foreign DNA and can infect mammalian cells, resulting in expression of a large amount of foreign protein. Fowlpox virus (FPV) is a member of the Poxviridae family (genus Avipoxvirus). Productive infection by fowlpox virus is restricted in vivo to avian species and in vitro to cells derived from avian species. However, inoculation of mammalian cells with avipox-based recombinants results in expression of foreign genes, and inoculation of mammals results in the induction of protective immunity.

Another embodiment provides an immunogenic composition based on modified vaccinia virus Ankara (MVA) expressing p28\textsuperscript{TEV} or an immunogenic fragment thereof. MVA is a highly attenuated strain of vaccinia virus that was developed toward the end of the campaign for the eradication of smallpox. Produced by hundreds of passages of vaccinia virus in chicken cells, MVA has lost about 10\% of the vaccinia genome and with it the ability to replicate efficiently in primate cells. Despite its limited replication, MVA provides similar levels of recombinant gene expression to those of replication-competent vaccinia viruses in human cells. Another embodiment provides an immunogenic composition based on non-replicating viral vectors made by deleting one or more genes from a virus capable of entering human cells. Nucleic acids encoding at least one p28\textsuperscript{TEV} polypeptide and optionally, one or more additional HIV antigens or cytokines replace the deleted viral gene resulting in a replication-incompetent viral vector. The antigens may be processed for presentation on the cell surface in association with MHC class I. Antigens presented in this way to CD8 T cells can elicit an HPV-specific cytotoxic T-cell response.

Representative non-replicating viral vectors include, but are not limited to, gene-deleted adenovirus constructs ("adenovectors") such as vectors derived from adenovirus type 5 (Ad5) which have been rendered replication-incompetent by deletion or inactivation of the E1 region (and in some cases other adenoviral genes as well). Adenovectors can contain deletions in multiple coding regions. The "missing" adenoviral gene products can be supplied by packaging cell lines specifically engineered to produce the vectors, but are not subsequently produced by the vectors themselves.

An embodiment provides an immunoegenic composition comprising at least one naked DNA or a naked RNA encoding at least one polypeptide according to the disclosure. Naked DNA or RNA is DNA or RNA that does not have proteins or lipids associated with it.

A representative combination immunogenic composition would include, for example, gp120 in combination with a nucleic acid encoding p28\textsuperscript{TEV}.
In an embodiment, the immunogenic composition includes a vector such as a plasmid that includes a gene encoding at least one p28<sup>TCV</sup> polypeptide and optionally, at least one additional HIV antigen and/or immunomodulator such as IL-2, under the transcriptional control of a promoter region active in human cells. In some embodiments, the vector does not encode at least one other HIV protein. The coding region of p28<sup>TEV</sup> is followed by transcription termination and polyadenylation sequences. To permit selection of plasmid-containing bacteria during the production process, the plasmid also contains an antibiotic resistance gene with a bacterial origin of replication. DNA is generally less costly to produce than peptide or protein, and is chemically stable under a variety of conditions. DNA is generally administered intramuscularly, using either a needle and syringe or a needle-free injector.

In another embodiment, at least one p28<sup>TEV</sup> polypeptide according to the disclosure makes up the composition of a lipoprotein or of a lipoprotein. The association of a p28<sup>TEV</sup> peptide with a fatty acid or phospholipid can potentiate an immunogenic response compared to the response caused by administering the p28<sup>TEV</sup> peptide antigen alone. The p28<sup>TEV</sup> compositions according to the disclosure may be coupled to microparticles or nanoparticles containing a polysaccharide core and/or covered in particular with a lipid bilayer. They may also be coupled to one or more liposomes or one or more niosomes (nonionic surfactant vesicles).

In some embodiments, an immunogenic composition can comprise at least one polynucleotide encoding a V1 peptide from the V1 region of p28<sup>TEV</sup>. In some embodiments, preferably, the polynucleotide does not encode a full length naturally occurring env polypeptide. In some embodiments, a V1 peptide corresponds to amino acids 75-103 of p28<sup>TEV</sup> of 89.6 (the sequence starting at the cysteine at position 75 and ending at the cysteine at position 103). (Figure 33) In some embodiments, the peptide comprises sequence of:

20 NLN ITKNNTNPTSSS (SEQ ID NO: 136),
21 TNKTNNPTSSSSWGMMM (SEQ ID NO: 137),
22 TNPTSSSSWGMMEKGE (SEQ ID NO: 138),
23 SSSWGMMEKGEIKNC (SEQ ID NO: 139), or
24 GMMEKGEIKNCFSFYI (SEQ ID NO: 140).

In other embodiments, the V1 polypeptide comprises an amino acid sequence selected from the group consisting of:

SSSWGMMEKGE (SEQ ID NO: 152),
SSSRGMVGGGE (SEQ ID NO: 153),
SSNWKEKMDRGE (SEQ ID NO: 154), and
SSSGRMIMEKGE (SEQ ID NO: 155).
In other embodiments, a V1 consensus sequence comprises a formula of contiguous amino acids comprising SSSX_4X_5MX_8X_9GE (SEQ ID NO: 156); wherein X4 is W, R, or G; X5 is G, K, or R; X8 is M, V or I; X9 is E, G, D, or M; and X9 is K, G, R, or E.

In other embodiments, an isolated and purified V1 polypeptide comprises at least one V1 consensus sequence of a clade as shown in Figure 35. In some embodiments, an isolated V1 consensus sequence comprises:

for clade A:

\[
\text{CSNX}_4\text{XsNNTX}_9\text{X}_{10}\text{XnNTNX}_{18}\text{TDGMREEKNC}
\]  
(SEQ ID NO: 160)

for clade B:

\[
\text{CTDLNNTNX}_8\text{s}_9\text{TSSGGTMEKGBKNC}
\]  
(SEQ ID NO: 161)

for clade C:

\[
\text{CTNVNINX}_{8}\text{TX}_{10}\text{X}_{11}\text{GX}_{18}\text{NTYNSMX}_{20}\text{X}_{21}\text{EIKNC}
\]  
(SEQ ID NO: 162)

for clade D:

\[
\text{CTDASRNX}_{8}\text{TXioXnNTNGPX}_{17}\text{MEKEMKNC}
\]  
(SEQ ID NO: 163)

for clade G:

\[
\text{CTNVNNX}_{8}\text{TX}_{10}\text{X}_{11}\text{NX}_{16}\text{TVX}_{20}\text{EEKNC}
\]  
(SEQ ID NO: 164)

for clade O:

\[
\text{CTNX}_{4}\text{Xs}_{5}\text{GTTX}_{10}\text{XnX}_{12}\text{X}_{13}\text{X}_{14}\text{X}_{15}\text{X}_{16}\text{X}_{17}\text{X}_{18}\text{ENLMKQC}
\]  
(SEQ ID NO: 165)

wherein an X amino acid is any of the 20 naturally occurring amino acids.

Another embodiment provides an immunogenic composition comprising p28TEV polypeptide or fragment thereof, or a p28TEV polynucleotide, at least one additional HIV antigen, a polynucleotide encoding an additional HTV antigen, at least one immunomodulator, or combinations thereof. Suitable additional HTV antigens include, but are not limited to Vif, Tat, Gag, Env, Rev, gpl20, gp41, p24, p7, pl7, combinations thereof as well as polypeptides encoded by gag, nef, pol, env, vpr, vpu, a combination thereof, or immunogenic fragments thereof.

In an embodiment, a subject is initially primed with a polynucleotide encoding p28TEV polypeptide in one vector. In some embodiments, the vector does not encode at least one other HTV polypeptide. In some embodiments, the host is subsequently dosed with a polynucleotide encoding a p28TEV polypeptide in a second vector which is different from the first vector. This approach has also been termed "heterologous boosting," to distinguish it from the traditional method (homologous boosting) in which two or more doses of the same immunogenic composition are given successively. Heterologous boosting helps to minimize immunogenic response to the vector itself. The dose used to boost the immune response can include one or more cytokines, chemokines, immunomodulators, or HIV antigens not present in the priming dose of the immunogenic composition. In a further embodiment,
the subject is subsequently boosted with one or more p28<sup>TEV</sup> polypeptides or fragment thereof, and optionally an immunomodulator or adjuvant.

In some embodiments, naturally occurring p28<sup>TEV</sup> polynucleotides may be isolated by cloning out virus from infected individuals and selectively amplifying polynucleotide region encoding p28<sup>TEV</sup> and expressing these polynucleotides to isolate naturally occurring p28<sup>TCV</sup> polypeptides present in infected individuals at different stages of infection. Primers for amplifying p28<sup>TEV</sup> sequences can be designed from the sequences provided in Figures 26-33. Such polynucleotides or polypeptides may be useful in the immunogenic compositions described herein.

B. p28<sup>TEV</sup> Polypeptides

HIV-I encodes alternatively spliced mRNAs for several proteins that regulate viral transcription and replication. Among them, two mRNAs that encode two proteins, p18 and p28<sup>TEV</sup>, have been described. Benko et al., 1990, <i>J. Virol</i>, 64:2505-2518. HIV-I p28<sup>TEV</sup> is encoded by alternatively spliced mRNA that joins together the first exon of Tat to a region with the Env (exon 6D) and to the second exon of Rev. This protein is expressed early in infection and in fact, is the first protein expressed during infection. Benko et al., 1990, <i>J. Virol</i>, 64:2505-2518.

The p28<sup>TEV</sup> is a phosphoprotein and is immunoprecipitated with both anti-tat and anti-rev monoclonal antibodies. Since humans develop antibodies to Tat and Rev, p28<sup>TEV</sup> likely is recognized by sera of HTV-I-infected humans. p28<sup>TEV</sup> has both tat and rev functions, although the rev function is not as strong as that of rev protein itself. The amino terminal of p28<sup>TEV</sup> has the first exon of tat and functional studies show that the first 58 amino acids of exon 1 are sufficient for full tat activity, p28<sup>TEV</sup> appears to have comparable activity to that of tat alone. The carboxy terminus of p28<sup>TEV</sup> has the second exon of rev including the domain that may act as a nuclear localization signal. The exon of the Env gene of p28<sup>TEV</sup> includes the V1 Env region of the virus, which is highly variable. Therefore, naturally occurring p28<sup>TEV</sup> polypeptides have amino acid sequence variability at least in the portion of the p28<sup>TEV</sup> polypeptide encoded by the V1 Env region. p28<sup>TEV</sup> is also found extracellularly. In an embodiment, an isolated and purified p28<sup>TEV</sup> polypeptide comprises an amino acid sequence of SEQ ID NO: 151. In other embodiments, an isolated and purified p28<sup>TEV</sup> polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 146, and SEQ ID NO: 151.

A comparison of HIV-I proviruses shows that exon 6D is conserved in some of the HIB family of HIV-I molecular clones that belong to clade B of HIV. Benko et al., 1990, <i>J. Virol</i>, 64:2505-2518. Other proviral clones lack one or both of the exon 6D splice sites, but may contain alternative splice sites in the vicinity of the exon 6D sequence (Benko et al., 1990, <i>J. Virol</i>, 64:2505-2518) such that proviral clones lacking exact matches for the exon
6D splice acceptor site and splice donor site may generate naturally occurring p28<sub>TEV</sub> variant polypeptides through recognition of other alternative splice sites in the Env gene.

V1 regions of p28<sub>TEV</sub> polypeptides of other HTV isolates corresponding to amino acids 75 to 103 of p28<sub>TCV</sub> of isolate 89.6 can be identified by finding the sequences and aligning them using the tools, for example, provided at GenBank or the Los Alamos database. In some embodiments, the V1 peptide does not comprise a full-length Env protein.

In other embodiments, an isolated and purified V1 polypeptide comprises at least one V1 consensus sequence of a clade as shown in Figure 35. In some embodiments, an isolated V1 consensus sequence comprises for clade A:

\[
\text{CSNX4XsNNTX}_gX_oX\alphaX\piNT}\text{JX},5\text{TDGMREEKNC}
\] (SEQ ID NO:160)
for clade B:

\[
\text{CTDLNNTNX}_9X_{10}TSSSGTMEKGEIKNC
\] (SEQ ID NO: 161)
for clade C:

\[
\text{CTNVNI\text{NX}}_8\text{TXioXnGXi3NTYNMX}_2\alphaX_{2}\text{iEIKNC}
\] (SEQ ID NO: 162)
for clade D:

\[
\text{CTDASRXN}_{8\text{TX}}(\alphaX_{11})\text{NTNGPXE}_{17}\text{MEKGMKNC}
\] (SEQ ID NO:163)
for clade G:

\[
\text{CTSVNNX}_{7X_{8X}9X_{1}X_{11}X_{13}X_{14}X_{18}X_{18}X_{17}X_{18}}\text{ENLMKQC}
\] (SEQ ED NO:165); wherein an X amino acid is any of the 20 naturally occurring amino acids.

Naturally occurring p28<sub>TEV</sub> polypeptides may be identified by antibodies that specifically bind Tat, Rev, and/or the V1 Env region, or by at least 70% sequence identity to an amino acid sequence comprising SEQ ID NO:151 (Figure 33). Preferably, naturally occurring p28<sub>TEV</sub> polypeptides have a biological activity of a p28<sub>TEV</sub> polypeptide reference sequence and is a polypeptide that is expressed early during infection. In a preferred embodiment, the biological activity is Tat transcriptional activity in a HIV-Luciferase assay as described herein.

C. p28<sub>TEV</sub> Polypeptide Variants

The disclosure also encompasses p28<sub>TEV</sub> polypeptide variants and immunogenic compositions containing them. p28<sub>TEV</sub> polypeptide variants can be prepared by introducing appropriate nucleotide changes into the p28<sub>TEV</sub> polypeptide encoding DNA, and/or by synthesis of the desired p28<sub>TEV</sub> polypeptide variant or isolated from naturally occurring sources. p28<sub>TCV</sub> polypeptides can be isolated and purified using methods such as affinity purification. p28<sub>TEV</sub> polypeptide variants may be useful as antagonists or to prepare antagonists.
A p28\textsuperscript{TEV} variant has at least about any one of the % of 70\% to 100 \% sequence identity to a full-length mature p28\textsuperscript{TEV} polypeptide reference sequence. A p28\textsuperscript{TEV} variant has at least about 70\% sequence identity, more preferably at least about 75\% sequence identity, more preferably at least about 80\% sequence identity, more preferably at least about 85\% sequence identity, more preferably at least about 90\% sequence identity, more preferably at least about 95\% sequence identity and even 100\% sequence identity to a full-length mature p28\textsuperscript{TEV} polypeptide reference sequence, such as a polypeptide having the sequence of SEQ ID NO: 151. Alternatively, a p28\textsuperscript{TEV} variant can have any number \% of about 70 to 100\% sequence identity to a reference peptide selected from the group consisting of SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, and SEQ ID NO: 156.

Preferably, the polypeptide variants increase Tat activity in the HIV-I-Luc assay. In some embodiments, the transcriptional activity is increased at least 2 fold, more preferably 2 to 5 fold compared to control. In one embodiment, the p28\textsuperscript{TEV} polypeptide is an isolated and purified polypeptide comprising an amino acid sequence of SEQ ED NO: 151 (Figure 33).

Variants include naturally occurring variants having the sequence of p28\textsuperscript{TEV} polypeptide isolated from nature from different HIV strains. Variations in the naturally occurring full-length p28\textsuperscript{TCV} polypeptides described herein, can also be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the p28\textsuperscript{TCV} polypeptide that results in a change in the amino acid sequence of the p28\textsuperscript{TEV} polypeptide as compared with a naturally occurring p28\textsuperscript{TEV} polypeptide.

Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the p28\textsuperscript{TEV} polypeptide with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Functional domains can also be identified in those p28\textsuperscript{TEV} polypeptides that have homology to known polypeptides.

Functional domains of p28\textsuperscript{TCV} are known. As described previously, p28\textsuperscript{TCV} includes a portion of the Tat protein, the V1 envelope and the Rev protein. The Tat activity of p28\textsuperscript{TCV} is similar to that of Tat, while the activity of the rev portion of p28\textsuperscript{TEV} is decreased compared to that of the rev protein. The amino terminal of p28\textsuperscript{TEV} has the first exon of Tat and functional studies show that the first 58 amino acids of exon 1 are sufficient for full Tat activity. p28\textsuperscript{TEV} appears to have comparable activity to that of tat alone. The carboxy terminus of p28\textsuperscript{TEV} has the second exon of rev including the domain that may act as a
nuclear localization signal. The exon of the Env gene of p28TEV includes the V1 Env region of the virus, which is highly variable. Therefore, naturally occurring p28TEV polypeptides may have amino acid sequence variability at least in the portion of the p28TEV polypeptide encoded by the V1 Env region. See Figure 35 to identify positions of V1 region that can be substituted without affecting function.

The sequences of these functional domains can be compared and aligned to other known sequences for Tat, V1 and Rev from other HIV-1 strains that may be provided at the Los Alamos website or GenBank, and locations of amino acid positions for substitutions can be identified as those positions that show a high degree of variability in amino acids, i.e., at least 3 different amino acids are found at that position when different sequences are aligned and compared or have a lower percentage of sequence identity i.e., less than 90% sequence identity. When sequences are aligned the positions that show variability can either have conservative amino acid substitutions or non-conservative amino acid substitutions. If the position has conservative amino acid substitutions that would indicate that the amino acid substituted at that position should be of the same type as those observed to be at that position in naturally occurring proteins. For examples of such substitutions, see Table 1.

For example, based on the known variability in the V1 region of the env, one or more amino acid substitutions can be made in this portion of p28TEV without affecting function.

Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature naturally occurring sequence. Preferably, variants have the biological activity of the source molecule, such as increased Tat activity in the Luc assay.

In particular embodiments, conservative substitutions of interest are shown in Table 1 under the heading of preferred substitutions.

<table>
<thead>
<tr>
<th>Residue</th>
<th>Exemplary Substitutions</th>
<th>Preferred Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>val; leu; ile</td>
<td>val</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>lys; gin; asn</td>
<td>lys</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>gin; his; lys; arg</td>
<td>gin</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>glu</td>
<td>glu</td>
</tr>
<tr>
<td>CyS (C)</td>
<td>ser</td>
<td>ser</td>
</tr>
</tbody>
</table>
GIc (Q) asn asn
GIu (E) asp asp
GIy (G) pro; ala ala
His (H) asn; gin; lys; arg arg
e (I) leu; val; met; ala; phe; norleucine
Ile (I) leu; val; met; ala; phe; norleucine
Leu (L) norleucine; ile; val; met; ala; phe ile
Lys (K) arg; gin; asn arg
Met (M) leu; phe; ile leu
Phe (F) leu; val; ile; ala; type leu
Pro (P) ala ala
Ser (S) thr thr
Thr (T) ser ser
Trp (W) tyr; phe tyr
Tyr (Y) trp; phe; thr; ser phe
Val (V) ile; leu; met; phe; ala; norleucine leu

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., *Nucl. Acids Res.*, 13:4331 (1986); Zoller et al., *Nucl. Acids Res.*, 10:6487 (1987)], cassette mutagenesis [Wells et al., *Gene*, 34:315 (1985)], restriction selection mutagenesis [Wells et al., *Philos. Trans. R. Soc. London Ser A*, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the p28<sub>TEV</sub> polypeptide variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically preferred among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, *Science*, 244: 1081-1085 (1989)].

Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, *The Proteins*, (W.H. Freeman & Co., N.Y.); Chothia, *J. Mol. Biol.*, 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

p28<sub>TEV</sub> polypeptide variants also include naturally occurring polypeptide variants. A reference p28<sub>TEV</sub> polypeptide is obtained from a single source of HTV-I, such as HXB2, and the sequence of this reference or source polypeptide may differ from the sequence of the same type of p28<sub>TEV</sub> polypeptide obtained from either a strain from the same clade or from
other strains from another clade. p28\textsuperscript{TEV} polypeptide variants may be identified using antibodies that specifically bind, for example, Tat, Rev, and/or the V1 Env region, or by having with sequence identity to an amino acid sequence of SEQ ID NO: 1. For example, p28\textsuperscript{TEV} can be identified by binding to an anti-V1 and an anti-tat or anti-rev antibody.

p28\textsuperscript{TEV} polypeptide fragments are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full-length protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity of the p28\textsuperscript{TEV} polypeptide.

In some embodiments, an immunogenic composition can comprise at least one isolated and/or purified V1 peptide from the V1 region of p28\textsuperscript{TEV}, preferably corresponding to amino acids 75-103 of p28\textsuperscript{TEV} of 89.6. In some embodiments, the peptide does not include tat, rev, and/or a naturally occurring full length tev or env polypeptide. In some embodiments, the peptide comprises a sequence of:

\[
\begin{align*}
20 & \text{NLN} \text{TTTTNTPSSS} & (\text{SEQ ID NO: 136}), \\
21 & \text{TKNTTTNTPSSSWGM} & (\text{SEQ ID NO: 137}), \\
22 & \text{TNPTSSSWGMEMKE} & (\text{SEQ ID NO: 138}), \\
23 & \text{SSSWGMEMKEIKN} & (\text{SEQ ID NO: 139}), \\
24 & \text{GMEMKEIKNC} & (\text{SEQ ID NO: 140}).
\end{align*}
\]

In other embodiments, the V1 polypeptide comprises an amino acid sequence selected from the group consisting of:

\[
\begin{align*}
\text{SSSWGMEMKE} & (\text{SEQ ID NO: 152}), \\
\text{SSSGMVGGG} & (\text{SEQ ID NO: 153}), \\
\text{SSNWKEKDRG} & (\text{SEQ ID NO: 154}), \text{and} \\
\text{SSSGRMIMEKE} & (\text{SEQ ID NO: 155}).
\end{align*}
\]

In other embodiments, a V1 consensus sequence comprises a formula of contiguous amino acids comprising SSSX\textsubscript{4}X\textsubscript{5}MX\textsubscript{7}X\textsubscript{8}X\textsubscript{9}GE (SEQ BDNO: 156); wherein X4 is W, R, or G; X5 is G, K, or R; X7 is M, Vor I; X8 is E,G,D, or M; and X9 is K,G,R, or E.

In other embodiments, an isolated and purified V1 polypeptide comprises at least one V1 consensus sequence of a clade as shown in Figure 35. In some embodiments, an isolated V1 consensus sequence comprises for clade A:

\[
\text{CSNX}_{10}X\textsubscript{8}NNTX_{9}X_{10}NNTNX_{15}TDGMREEKNC \quad (\text{SEQ ID NO:160})
\]

for clade B:

\[
\text{CTDLNNTNX}_{9}X_{10}TSSSGTMEKEIKN \quad (\text{SEQ ID NO: 161})
\]

for clade C:

\[
\text{CTNVNTNX}_{9}X_{10}N\text{TYSNMX}_{20}X_{2}EIKN \quad (\text{SEQ ID NO: 162})
\]

for clade D:

\[
\text{CTDASRNX}_{9}X_{10}X_{1}X_{1}N\text{TNGPX}_{17}MEKEMKNC \quad (\text{SEQ ID NO: 163})
\]

\[
\text{CTDLNNTNX}_{9}X_{10}TSSSGTMEKEIKN \quad (\text{SEQ ID NO: 161})
\]

for clade C:

\[
\text{CTNVNTNX}_{9}X_{10}N\text{TYSNMX}_{20}X_{2}EIKN \quad (\text{SEQ ID NO: 162})
\]

for clade D:

\[
\text{CTDASRNX}_{9}X_{10}X_{1}X_{1}N\text{TNGPX}_{17}MEKEMKNC \quad (\text{SEQ ID NO: 163})
\]

\[
\text{CTDLNNTNX}_{9}X_{10}TSSSGTMEKEIKN \quad (\text{SEQ ID NO: 161})
\]

for clade C:

\[
\text{CTNVNTNX}_{9}X_{10}N\text{TYSNMX}_{20}X_{2}EIKN \quad (\text{SEQ ID NO: 162})
\]

for clade D:

\[
\text{CTDASRNX}_{9}X_{10}X_{1}X_{1}N\text{TNGPX}_{17}MEKEMKNC \quad (\text{SEQ ID NO: 163})
\]
for clade G:
CTNVN|VJX_8X_9X_10X_11NX_12TVTX_20EEK (SEQ ID NO:164)
for clade O:
CTNX_5GTTX_10XnX_12X_i_3Xi_18XcXnX_8ENLMkQC (SEQ ID NO:165);

wherein an X amino acid is any of the 20 naturally occurring amino acids.

In some embodiments, one or more of the V1 peptides may be combined with a heterologous molecule such as a short peptide that could provide for maintenance of a loop structure. For example, providing a short peptide of 1 to 5 amino acids on both ends of the peptide including a cysteine would allow formation of a disulfide bond which would provide a loop of each of the V1 peptides. Alternatively, a portion of the V1 region of p28TEV could be retained in order to provide for the loop structure of the V1 peptides, especially including the cysteines at positions 75 and 103 of 89.6 tev.

In some embodiments, the V1 peptides may be naturally occurring variants corresponding to the same region of the peptides 20-24 shown above. In other embodiments, variants of the peptides 20-24 can be derived using standard methods. Variants can have any number % sequence identity of about 70 to 100% sequence identity to a reference sequence such as that of SEQ ID NOS:136-140 or SEQ ID NOS:152-156. In addition, an immunogenic composition comprises one or more peptides corresponding to the peptide of SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, or SEQ ID NO:156 from one or more HIV clades. In another embodiment, an immunogenic composition may comprise one or more consensus sequences for the V1 portion of p28TEV or corresponding to the V1 peptides described herein.

The disclosure also contemplates immunogenic fragments of p28TEV polypeptides. Immunogenic fragments are at least 8 amino acids in length, more preferably 8-50 amino acids, more preferably at least 10 amino acids, and more preferably at least 20 amino acids up to a full-length polypeptide. Immunogenic fragments can be prepared synthetically, recombinantly, or by enzymatic digestion of p28TEV polypeptide. Immunogenic fragments can be predicted by analyzing the primary amino acid sequence of a p28TEV polypeptide using commercially available services such as Epipredict or Epitope informatics or publicly available programs such as are available. The fragments can be used to select or generate an antibody that specifically binds to a p28TEV polypeptide.

p28TEV polypeptide fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating p28TEV polypeptide fragments by enzymatic digestion, e.g., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and
isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5’ and 3’ primers in the PCR. Preferably, p28TEV polypeptide fragments share at least one biological and/or immunological activity with p28TEV polypeptide comprising an amino acid sequence of SEQ ID NO: 151.

Fusion proteins of the polypeptides or peptides described herein can also be prepared. The fusion protein can be attached to a heterologous moiety to provide a detectable label, increase immunogenicity or increase half life. Detectable labels include radionuclides, dyes, biotin, flag tag and the like. Polypeptides that increase immunogenicity include toxoids, glutathione reductase, albumin, and the like. Moieties that increase half life include polyethylene glycol.

D. Nucleic Acids

A second aspect of the disclosure relates to polynucleotides encoding p28TEV polypeptides, recombinant vectors, and host cells containing the recombinant vectors, as well as methods of making such vectors and host cells by recombinant methods. The polynucleotides encoding p28TEV or p28TEV variants are useful as immunogenic compositions, to produce p28TEV polypeptides or to prepare antagonists of p28TEV expression. In some embodiments, the polynucleotide does not encode at least one or more other HTV polypeptides.

The p28TEV polynucleotides of the disclosure may be synthesized or prepared by techniques well known in the art. See, for example, Creighton, Proteins: Structures and Molecular Principles, W. H. Freeman & Co., New York, NY (1983). Nucleotide sequences encoding the p28TEV polypeptides of the disclosure may be synthesized, and/or cloned, and expressed according to techniques well known to those of ordinary skill in the art. See, for example, Sambrook, et al., Molecular Cloning, A Laboratory Manual, VoIs. 1-3, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989).

The polynucleotides may be produced by standard recombinant methods known in the art, such as polymerase chain reaction (Sambrook, et al., 1989, Molecular Cloning, A Laboratory Manual, VoIs. 1-3, Cold Spring Harbor Press, Cold Spring Harbor, NY), or the DNA can be synthesized and optimized for expression in bacteria or eukaryotic cells. Primers can be prepared using the polynucleotide sequences provided, for example, in figures 26-33 or that are available in publicly available databases. The polynucleotide constructs may be assembled from polymerase chain reaction cassettes sequentially cloned into a vector containing a selectable marker for propagation in a host. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in E. coli and other bacteria.
Representative examples of appropriate hosts include, but are not limited to, bacterial cells such as E. coli, Streptomyces and Salmonella typhirium, fungal cells such as yeast; insect cells such as Drosophilia S2 and Spodoptera Sf9, animal cells such as CHO, 293T, COS, and Bowes melanoma cells, and plant cells. Appropriate culture medium and conditions for the above-described host cells are known in the art.

The polynucleotide can be operably linked to an appropriate promoter, such as CMV or the T7 promoter. Other suitable promoters are known in the art. The expression constructs may further contain sites for transcription initiation, transcription termination, and a ribosome binding site for translation. The coding portion of the mature polypeptide expressed by the constructs preferably includes a translation initiating codon at the beginning and a termination codon (UAA, UGA, or UAG) appropriately positioned at the end of the polypeptide to be translated.

Introduction of the recombinant vector into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in standard laboratory manuals such as Sambrook, et al., 1989, Molecular Cloning, A Laboratory Manual, VoIs. 1-3, Cold Spring Harbor Press, Cold Spring Harbor, NY or Davis et al., 1986, Basic Methods in Molecular Biology. Commercial transfection reagents, such as Lipofectamine (Invitrogen, Carlsbad, CA), Effectene (Qiagen, Valencia, CA) and FuGENE 6™ (Roche Diagnostics, Indianapolis, IN), are also available.

The polypeptide, or fragment thereof, may be expressed in a modified form, such as a fusion protein, and may include secretion signals and/or additional heterologous functional regions. For example, a region of additional amino acids may be added to the N-terminus or C-terminus of the polypeptide to facilitate detection or purification, improve immunogenicity, improve half-life, or improve persistence in the host cell during, for example, purification or subsequent handling and storage. Examples of additional amino acids include peptide tags that may be added to the polypeptide to facilitate detection and/or purification. Such peptide tags include, but are not limited to, His, HA, Avi, biotin, c-Myc, VSV-G, HSV, V5, or FLAG™. Examples of a polypeptide that can enhance immunogenicity include bovine serum albumin, and/or keyhole lymphocyte hemocyanin. Examples of molecules that improve half life include polyethylene glycol.

The polypeptide can be recovered and purified from recombinant cell cultures by methods known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography, and lectin chromatography. In an embodiment, high performance liquid chromatography (HPLC) is employed for purification.
The disclosure also includes variants of a polynucleotide encoding a p28^{TEV} protein having a sequence of SEQ ID NO: 151. In still another embodiment, the polypeptide is selected from the group consisting a polypeptide comprising the amino acid sequence of SEQ ID NO: 1 SEQ ID NO: 143, SEQ ID NO: 146, and SEQ ID NO: 151. These variant polynucleotide sequences may encode naturally occurring variants of p28^{TEV} obtained from different HIV-I strains or clades. The variants may also be made by substitution of nucleotides using standard methods.

Naturally occurring polynucleotides encoding variants of p28^{TEV} polypeptides can be isolated from cloning out viral isolates from infected individuals at various times post infection. Such polynucleotides can be obtained using primers for amplifying polynucleotide encoding p28^{TEV}. Such polynucleotides or polypeptides may be utilized in the immunogenic compositions described herein.

The disclosure also includes variants of nucleic acid molecules encoding p28^{TEV} polypeptides. In some embodiments, the disclosure includes polynucleotides having at least about 70% sequence identity, more preferably about 75% sequence identity, more preferably about 80% sequence identity, more preferably about 85% sequence identity, more preferably about 90% sequence identity, more preferably about 95% sequence identity, and even up to 100% sequence identity to a polynucleotide sequence encoding a p28^{TEV} protein having an amino acid sequence of SEQ ID NO: 151. In some embodiments, the polynucleotide variants encode a polypeptide that has a biological activity of p28^{TCV} of increased Tat activity in a Luc assay.

In some embodiments, the disclosure includes polynucleotides encoding a polypeptide having at least about 70% sequence identity, more preferably about 75% sequence identity, more preferably about 80% sequence identity, more preferably about 85% sequence identity, more preferably about 90% sequence identity, more preferably about 95% sequence identity, and even up to 100% sequence identity to a polypeptide sequence having an amino acid sequence of SEQ ID NO: 151. In still another embodiment, the reference polypeptide is selected from the group consisting a polypeptide comprising the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 146, SEQ ID NO: 151, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ EDNO: 139, SEQ IDNO: 140, SEQ ED NO: 152, SEQ ID NO: 153, SEQ ED NO: 154, SEQ ID NO: 155, and SEQ ED NO: 156. In some embodiments, the polynucleotide variants encode a polypeptide that has a biological activity of p28^{TEV} of increased transcriptional activity in a Luc assay.

Polynucleotide variants include variants made at the splice acceptor site in the Env region at position 6154 and/or the splice donor site in the Env region at position 6269 as shown in Figures 7 and 8 and/or other cryptic acceptor splice sites or donor splice sites in p28^{TCV} encoding DNA or mRNA. Targeting this splice acceptor site splice and/or donor
splice site by reprogramming alternative pre-mRNA splicing may prevent alternative splicing of p28<sub>TEV</sub> and allow the virus to be in switched on mode (e.g., preferentially express Tat and replicate), making it more accessible to the immune system. Sazani et al., 2002, <i>Nat.</i> Acids. Res., 29:3965-3974; Sanzani et al., 2002, <i>Nature Biotechnol.</i>, 20:1228-1233; Villemaire et al., 2003, <i>J. Biol. Chem.</i>, 278:5003 1-50039.

Vectors that are useful for expression of the polynucleotides of the disclosure include plasmid vectors as well as viral vectors. Several viral vectors are known to be useful in transducing DNA and/or RNA, such as lentivirus-based vectors.

The nucleic acids disclosed herein are useful in immunogenic compositions as described herein.

**E. Nucleic Acid Antagonists of p28<sub>TEV</sub> expression**

Novel therapeutics can be identified that can inhibit the expression and/or activity of these p28<sub>TEV</sub> polypeptides. Inhibition of the activity and/or expression of the p28<sub>TEV</sub> polypeptide may inhibit HTV viral levels of some isolates.

Agents that can act therapeutically to inhibit the effect of p28<sub>TEV</sub> on HIV-I viral levels include p28<sub>TEV</sub> variants, antagonist antibodies, antisense RNA specific for p28<sub>TEV</sub>, small interfering RNA (siRNA), microRNA or methods to modulate pre-mRNA splice site choice. In some embodiments, antagonists are targeted to inhibit p28<sub>TEV</sub> expression. The expression level of p28<sub>TCV</sub> can be measured by transcriptional activity using HIV luciferase or by reverse transcriptase activity.

Antisense RNA can be prepared based on the nucleic acid sequence encoding p28<sub>TEV</sub>, for example, SEQ ID NO:2. The p28<sub>TEV</sub> mRNA can be specifically eliminated by introduction or expression of antisense sequences targeted to the unique p28<sub>TEV</sub> sequences. Preferably, the antisense RNA is complementary to and/or binds under stringent conditions to a portion of the nucleic acid sequence including the splice acceptor site or splice donor site unique to p28<sub>TEV</sub>.

The location of the acceptor and donor sites of exon 6D in HXB2 mRNA is shown in Figures 7 and 8 and has been described in Benko et al. cited supra. In one embodiment, the splice acceptor site is found at the 5′ end of exon 6D of the V1 region of envelope protein of HXB2 (e.g., nucleotide 6154 of mRNA of HXB2, see Figure 7) and has a sequence of (A/C/U)AG as underlined in Figure 7 (SEQ ID NO:3). The donor splice site is found at the 3′ end of exon 6D of the V1 region of envelope protein of HXB2 (e.g., nucleotide 6269 of mRNA of HBX2, see Figures 8 and 10) and has a sequence of (A/CZG)AG as underlined in Figure 8 (SEQ ID NO:4). The nucleotides in the parenthesis represent alternative nucleotides that can be found at the same position. Preferably, the acceptor splice site and donor splice site have a nucleotide sequence comprising a conserved AG or AC. In some embodiments, the conserved nucleotides are flanked 5′ and/or 3′ by
one to three nucleotides that contribute to effective splicing. In some embodiments, the acceptor splice site comprises AGG or CAG or UAG (see Figure 7). In some embodiments, the donor splice site comprises AGG, CAG, or GAG (see Figure 8).

The locations of donor and acceptor splice sites in the DNA sequence corresponding to the mRNA are shown in Figures 7 and 8. The nucleotide numbers refer to the location of the splice sites in the mRNA. The numbering on the DNA sequence does not match that of the splice sites on the mRNA because the numbering of the DNA includes the 5' LTR sequence. The DNA sequences surrounding the donor and acceptor splice sites for exon 6D from representative HIV strains are aligned. The location of the donor and acceptor site sequences in the mRNA in the corresponding DNA sequences are shown; acceptor site in Figure 7 and donor in Figure 8. An examination of the DNA sequences at the location of the splice site sequences indicates these sequences are conserved among many different isolates, especially the donor site. One of skill in the art can identify additional acceptor splice sites and donor splice sites by aligning HIV-1 DNA sequences from the location surrounding the splice site sequences against the reference HXB2 sequence as shown in Figures 7 and 8.

In other embodiments, the anti-sense RNA is complementary to the acceptor splice site and about 20 to about 70 nucleotides upstream of the acceptor splice site that contribute to effective splicing. In other embodiments, the anti-sense RNA is complementary to the donor splice site and about 20 to about 70 nucleotides downstream of the donor splice site that contribute to effective splicing. In other embodiments, the anti-sense RNA is complementary to the donor or acceptor splice site and about 20 to about 70 nucleotides on either side of the site.

Antisense RNA may be prepared by any method known in the art for synthesis of DNA and RNA molecules. These methods include techniques for chemically synthesizing oligoribonucleotides, such as solid phase phosphoramidite chemical synthesis and recombinant techniques. Antisense RNA may be generated by in vitro or in vivo transcription of DNA sequences. (U.S. 2003/0220287) DNA sequences encoding the antisense RNA may be incorporated into a variety vectors known in the art that incorporate suitable RNA polymerase promoters. Alternatively, antisense cDNA constructs that synthesize the antisense RNA molecule constitutively or inducibly may be introduced stably into a cell. As a means of increasing intracellular stability and half-life, modifications may be made to the antisense RNA molecule. Such modifications include, but are not limited to, the addition of flanking sequences of ribonucleotides to the 5' and/or 3' end of the molecule.

Antisense constructs are delivered, for example, as an expression plasmid that when transcribed in the cell produces antisense RNA that is complementary to at least a unique portion of the cellular mRNA that encodes p28TEV, preferably the splice acceptor site. In an
embodiment, the antisense RNA molecule comprises a ribonucleotide sequence that is complementary to a specific p28^TEV mRNA splice junction. In one embodiment, the splice acceptor site is found at the 5' end of exon 6D of the V1 region of envelope protein of HXB2 (e.g. nucleotide 6154 of m RNA of HXB2, see Figures 7 and 8) and has a sequence of (A/C/U)AG as underlined in Figure 7 (SEQ ID NO:3). The donor splice site is found at the 3' end of exon 6D of the V1 region of envelope protein of HXB2 (e.g. nucleotide 6269 of m RNA of HBX2, see Figures 8 and 10) and has a sequence of (A/C/G)AG as underlined in Figure 8 (SEQ ID NO:4). The nucleotides in the parenthesis represent alternative nucleotides that can be found at the same position. Preferably, the acceptor splice site and donor splice site have a nucleotide sequence comprising a conserved AG or AC. In some embodiments, the conserved nucleotides are flanked 5' and/or 3' by one to three nucleotides that contribute to effective splicing. In some embodiments, the acceptor splice site comprises AGG or CAG or UAG (see Figure 7). In some embodiments, the donor splice site comprises AGG, CAG, or GAG (see Figure 8).

There is no particular limitation in the length of antisense RNA. See U.S. 2003/0220287. The length of antisense RNA is dependent upon the number and composition of complementary bases and the accessibility of the target sequence. (U.S. 2003/0220287) In one embodiment, antisense molecules are preferably 12-20 nucleotides. Optimal lengths of anti-sense molecules are known to those of skill in the art. There can be some mismatch in the anti-sense RNA as long as the antisense molecules binds to the target nucleotide sequence under stringent conditions, preferably under moderately stringent conditions and more preferably under highly stringent conditions.

Similarly, p28^TCV RNA levels can be depleted by introduction and/or expression of short interfering RNA (siRNA) designed to target the unique splice junctions present in the p28^TEV mRNA. siRNA induces gene-specific suppression through sequence specific degradation of homologous gene transcripts. P. Sharp, 1999, *Genes & Development*, 13:139-141; Berstein et al., 2000, *RNA*, 97:4985; U.S. 2003/0220287. For example, the antisense RNA strand of the siRNA is complementary to a portion of the nucleic acid sequence including the splice acceptor site or splice donor site unique to p28^TEV.

In one embodiment, the splice acceptor site is found at the 5' end of exon 6D of the V1 region of envelope protein of HXB2 (e.g. nucleotide 6154 of m RNA of HXB2, see Figures 7 and 8) and has a sequence of (A/C/U)AG as underlined in Figure 7 (SEQ ID NO:3). The donor splice site is found at the 3' end of exon 6D of the V1 region of envelope protein of HXB2 (e.g. nucleotide 6269 of m RNA of HBX2, see Figures 8) and has a sequence of (A/C/Z/G)AG as underlined in Figure 8 (SEQ ID NO:4). The nucleotides in the parenthesis represent alternative nucleotides that can be found at the same position. Preferably, the acceptor splice site and donor splice site have a nucleotide sequence
comprising a conserved AG or AC. In some embodiments, the conserved nucleotides are flanked 5' and/or 3' by one to three nucleotides that contribute to effective splicing. In some embodiments, the acceptor splice site comprises AGG or CAG or UAG (see Figure 7). In some embodiments, the donor splice site comprises AGG, CAG, or GAG (see Figure 8).

One of skill in the art can readily identify additional acceptor splice sites and donor splice sites by aligning HIV-1 DNA sequences against a reference sequence HXB2, as shown in Figures 7 and 8.

In other embodiments, the anti-sense strand of siRNA is complementary to the acceptor splice site and about 20 to about 70 nucleotides upstream of the acceptor splice site that contribute to effective splicing. In other embodiments, the anti-sense RNA is complementary to the donor splice site and about 20 to about 70 nucleotides downstream of the donor splice site that contribute to effective splicing. In other embodiments, the anti-sense RNA is complementary to the donor or acceptor splice site and about 20 to about 70 nucleotides on either side of the site.

siRNA may be prepared by any method known in the art for synthesis of DNA and RNA molecules, including chemical synthesis and recombinant techniques. (U.S. 2004/0002077; U.S. 2003/0220287) siRNA may be generated by in vitro or in vivo transcription of DNA sequences.

DNA sequences encoding the siRNA may be incorporated into a variety vectors known in the art that incorporate suitable RNA polymerase promoters. siRNA can be introduced in cultured cells either transiently or via stable expression from tetracycline inducible promoters. Efficient introduction of siRNA molecules into cells in vitro may be performed using a number of technologies, including lipid-based transfection techniques. siRNA can also be expressed in tissues or whole organs using viral expression systems known in the art that incorporate suitable RNA polymerase promoters. Preferably the expression system is introduced into the target cells using a vector that efficiently transfers the expression system. Vectors are selected depending on the type of cell to be transfected. Useful viral vectors include retrovirus vector, adenovirus vector, adeno-associated virus vector, vaccinia virus vector, lentivirus vector, herpesvirus vector, alphavirus vector, EB virus vector, papilloma virus vector, and foamyvirus vector. Useful non-viral vectors include cationic liposome, ligand DNA complex, and gene gun.

siRNA constructs are delivered, for example, as an expression plasmid that when transcribed in the cell produces siRNA comprising an antisense stand that is complementary to at least a unique portion of the cellular mRNA that encodes p28TEV. In an embodiment, the siRNA molecule comprises an antisense strand comprising a ribonucleotide sequence that is complementary to a specific p28TEV mRNA splice junction.
There is no particular limitation in the length of siRNA. See U.S. 2003/0220287. The siRNA may be 15 to 50 bp long, preferably 15 to 35 bp, and more preferably 21 to 30 bp. The double-stranded RNA portions of siRNAs may contain nonpairing nucleotides due to mismatches, wherein the corresponding nucleotides are not complementary, and/or bulges, wherein the corresponding complementary nucleotide is lacking on one strand. See U.S. 2004/0002077. Double stranded RNA (dsRNA) may comprise nonpairing nucleotides to the extent the nonpairing nucleotides do not interfere with siRNA formation. There can be some mismatch in the anti-sense RNA strand of the siRNA as long as the antisense strand binds to the target nucleotide sequence under stringent conditions, preferably under moderately stringent conditions and more preferably under highly stringent conditions.

p28\textsuperscript{TRV} production may also be inhibited by modulating pre-mRNA splice site choice. Short, chemically modified antisense oligonucleotides can be targeted to the specific splice junctions present in p28\textsuperscript{TRV}. These chemically modified antisense oligos, including but not limited to 2'-o-methyl-phosphoribonucleotides, morpholinos and PNA\textsubscript{s} (protein nucleic acids) act in a highly specific fashion by binding to their target sequence without inducing the RNase H degradation pathway typical for traditional antisense oligonucleotides. Binding of these reagents to the splice site prevents association of the pre-mRNA splicing machinery and thus leads to skipping of the targeted exon. In an embodiment, the targeted exon is exon 6 of p28\textsuperscript{TRV}. Anti-sense directed control of splicing is highly specific and efficient and is currently explored in clinical trials (Kalbfuss et al., 2001, JBC, 276:42986-93; Sazani and Kole, 2003, J. Clin. Invest., 112:481-6).

Oligonucleotide-protein chimeras may be used to target specific splice junctions (Cartegni et al., 2003, Nature Struct. Biol., 10:120-5). The oligonucleotide moiety targets the chimera to the appropriate exon, whereas the protein moiety consisting of minimal SR protein splicing factors stimulates formation of the spliceosome and thus enhances inclusion of the targeted splice site. Splice site junctions may also be targeted \textit{in vivo} by trans-splicing systems (Deidda et al., 2003, Nature Struct. Biol., 12:1499-1504).

In an embodiment, the antisense oligonucleotide comprises a nucleic acid sequence that is complementary to a specific p28\textsuperscript{TRV} mRNA splice junction. In one embodiment, the splice acceptor site is found at the 5' end of exon 6D of the V1 region of envelope protein of HXB2 (e.g. nucleotide 6154 of mRNA of HXB2, see Figures 7 and 8) and has a sequence of (A/C/U)AG underlined in Figure 8 (SEQ ID NO:3). The donor splice site is found at the 3' end of exon 6D of the V1 region of envelope protein of HXB2 (e.g. nucleotide 6269 of mRNA of HBX2, see Figure 7 and 9) and has a sequence of (A/C/G)AG underlined in Figure 9 (SEQ ID NO:4). The nucleotides in the parenthesis represent alternative nucleotides that can be found at the same position. Preferably, the acceptor splice site and donor splice site have a nucleotide sequence comprising a conserved AG or AC. In some embodiments, the
conserved nucleotides are flanked 5' and/or 3' by one to three nucleotides that contribute to effective splicing. In some embodiments, the acceptor splice site comprises AGG or CAG or UAG (see Figure 7). In some embodiments, the donor splice site comprises AGG, CAG, or GAG (see Figure 8).

In other embodiments, the anti-sense RNA is complementary to the acceptor splice site and about 20 to about 70 nucleotides upstream of the acceptor splice site that contribute to effective splicing. In other embodiments, the anti-sense RNA is complementary to the donor splice site and about 20 to about 70 nucleotides downstream of the donor splice site that contribute to effective splicing. In other embodiments, the anti-sense RNA is complementary to the donor or acceptor splice site and about 20 to about 70 nucleotides on either side of the site.

F. Compositions of p28\textsuperscript{TEV} Polypeptides

The p28\textsuperscript{TEV} polypeptides of the present disclosure are useful, for example, in immunogenic compositions, as immunogens for purifying anti-HIV antibodies from sera, for identifying and/or purifying anti-HIV monoclonal antibodies, in a method to screen for HIV, and in a method to identify antagonists of p28\textsuperscript{TEV} polypeptides.

The p28\textsuperscript{TEV} polypeptides of the disclosure can be employed in immunogenic compositions. Immunogenic compositions are useful to elicit p28\textsuperscript{TEV} antibodies and/or to inhibit HIV viral levels. These antibodies are useful, for example, in diagnostic assays to screen for HIV infection and as therapeutic antagonists of p28\textsuperscript{TEV} activity.

The immunogenic composition preferably comprises at least one isolated and/or purified p28\textsuperscript{TEV} polypeptide or immunogenic fragment thereof and more preferably, comprises a mixture of naturally occurring p28\textsuperscript{TEV} polypeptides from at least about 2 to about 6 different HIV-I clades, more preferably about 3 to about 6 different HIV-I clades. Because of the variability of the V1 portion of p28\textsuperscript{TEV}, it may be necessary to include p28\textsuperscript{TEV} polypeptides from many different clades.

In some embodiments, the immunogenic composition comprises at least one isolated and/or purified naturally occurring polynucleotide encoding a p28\textsuperscript{TEV} polypeptide or p28\textsuperscript{TEV} polypeptides isolated from infected individuals at various times post infection.

Naturally occurring HIV isolated from an individual can be cloned and polynucleotides encoding p28\textsuperscript{TEV} isolated and characterized. The p28\textsuperscript{TCV} polynucleotides or polypeptides so isolated can be utilized to prepare a specific therapeutic or immunogenic composition for an individual.

In some embodiments, an immunogenic composition can comprise at least one isolated V1 peptide from the V1 region of p28\textsuperscript{TEV}, preferably corresponding to amino acids 75-103 of p28\textsuperscript{TEV} of 89.6. In some embodiments, the peptide does not comprise a full length
env polypeptide. In some embodiments, the peptide from the V1 region corresponds to one or more of the peptides 20-24. In some embodiments, the peptide comprises a sequence of:

(20)  NLTNKTNTNPTSSS  (SEQ ID NO: 136)
(21)  TKNNTNPTSSSWGMM  (SEQ ID NO: 137)
(22)  TNPTSSSWGMMEKGE  (SEQ ID NO: 138)
(23)  SSSWGMMMEKGEIKNC  (SEQ ID NO: 139) or
(24)  GMMEKGEKNCFSFYI  (SEQ ID NO: 140).

In other embodiments, the V1 polypeptide comprises an amino acid sequence selected from the group consisting of:

10  SSSWGMMMEKGE  (SEQ ID NO: 152),
    SSSRGMVGGGE  (SEQ ID NO: 153),
    SSNWKEMDRGE  (SEQ ID NO: 154), and
    SSSGRMIMEKG  (SEQ ID NO: 155).

In other embodiments, a V1 consensus sequence comprises a formula of contiguous amino acids comprising SSSX₄X₅MX₇X₈X₉GE (SEQ IDNO: 156); wherein X₄ is W, R, or G; X₅ is G, K, or R; X₇ is M, Vor I; X₈ is E,G,D, or M; and X₉ is K,G,R, or E. These peptides can be used to generate or select antibodies specific for p28ENV polypeptide. In some embodiments, the immunogenic composition comprises one or more V1 sequences from a HIV isolate of a specific clade. In some embodiments, consensus sequences of the V1 region for each clade are included in the immunogenic composition. In some embodiments, the V1 polypeptide does not comprise a full-length TeV or Env protein.

In other embodiments, an isolated and purified V1 polypeptide comprises at least one V1 consensus sequence of a clade as shown in Figure 35. In some embodiments, an isolated V1 consensus sequence comprises for clade A:

25  CSNX₄X5NNTX9X10X11NTNX15TDGMREEKNC  (SEQ ID NO: 160)

for clade B:

CTDLNNTNXsX₅oTSSSGTMEKGEIKNC  (SEQ ID NO: 161)

for clade C:

CTNVNINXgTXioX₇GXisNTYNSMXaoXaiEIKNC  (SEQ ID NO:162)

for clade D:

CTDASRNX₈TX₁₀XnNTNGPX₁₇MEKGMKNC  (SEQ ID NO:163)

for clade G:

CTNVNNX₇X₈X₉X₁₀X₁₁NTX₁₃NTNX₁₅TVTX₂₀EEKNC  (SEQ ID NO: 164)

for clade O:

35  CTNX₄X₅GTTX₉X₁₀XnX₁₂X₁₃X₁₄X₁₅X₁₆X₁₇X₁₈X₁₉X₂₀X₂₁X₂₂X₂₃X₂₄X₂₅ENLMKQC  (SEQ ID NO: 165);

wherein an X amino acid is any of the 20 naturally occurring amino acids.
The p28^TEV polypeptides are preferably present in an immunogenic effective amount. An immunogenic effective amount is an amount of P28^TEV polypeptide that induces an immune response in an animal. The actual amount of the polypeptide may vary depending on the animal to be immunized, the route of administration and adjuvants.

Immunogenic dosages can be determined by those of skill in the art. The immune response may be indicated by T and/or B cell responses. Typically, the immune response is detected by the presence of antibodies that specifically bind to a particular p28^TEV polypeptide. Methods of detecting antibodies top28^TEV polypeptides are known to those of skill in the art and include such assays as ELISA assays, ELISPOT assays, western blot assays, and competition assays.

In one embodiment, animals are immunized with the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 µg or 5 µg of the protein or conjugate with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/2 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

The compositions of the disclosure also include a carrier. Carriers include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™ polyethylene glycol (PEG), and PLURONICS™.

The polypeptides of the disclosure can be administered orally or parentally, including subcutaneous injection, intravenous, intramuscular, intratrernal or infusion techniques, in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants or vehicles. Compositions of the disclosure can be in the form
of suspensions or tablets suitable for oral administration or sterile injectable preparations, such as sterile injectable aqueous or oleagenous suspensions.

For administration as injectable solutions or suspensions, the compositions can be formulated according to techniques well-known in the art, using suitable dispersing or wetting and suspending agents, such as sterile oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

G. Antibodies

The p28<sub>TEV</sub> polypeptides of the disclosure are immunogenic and elicit anti-p28<sub>TEV</sub> polypeptide antibodies. The antibodies have many uses, including purifying p28<sub>TCV</sub> polypeptide of the disclosure, detecting HIV, targeting HIV infected cells, and/or inhibiting viral levels. The antibodies may be polyclonal or monoclonal antibodies.

The antibody may be used to detect HTV-infection in a fluid or tissue from a subject. The antibody typically will be labeled with a detectable moiety including, but not limited to, a fluorescent label, a radioisotope, or an enzyme-substrate label. The label may be indirectly conjugated with the antibody. For example, the antibody can be conjugated with biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin, or vice versa. Biotin binds selectively to avidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody, the antibody is conjugated with a small hapten (e.g., digoxin) and one of the different types of labels mentioned above is conjugated with an anti-hapten antibody (e.g., anti-digoxin antibody).

In another embodiment of the disclosure, the antibody does not need to be labeled. The antibody is detected using a labeled antibody that binds to the first antibody.

Antibodies to p28<sub>TEV</sub> can be screened for antagonist activity. Preferably, antibodies to the p28<sub>TEV</sub> polypeptide inhibit HIV viral levels. An antagonist antibody would be screened to determine if there was decrease of viral infectivity or viral load in infected cells expressing p28<sub>TEV</sub>. Alternatively, the antibodies could be screened for effects on the p28<sub>TEV</sub> transcriptional activity. In another embodiment, antibodies are selected that participate in antibody dependent cytotoxicity of HIV infected cells.

In some embodiments, the antibody specifically binds to the V1 region of p28<sub>TEV</sub>. Li some embodiments, antibodies specific for V1 region of p28<sub>TEV</sub> do not crossreact with Tat or Rev region of p28<sub>TEV</sub>. These antibodies may be useful as therapeutic agents or as targeting agents in combination with an antagonist such as siRNA or cytotoxic agents.

In an embodiment, the antibodies specifically bind p28<sub>TEV</sub> and do not crossreact with Tat and/or Rev. Screening methods for identifying antibodies that bind to p28<sub>TEV</sub> and do not bind to Tat and/or Rev are known to those of skill in the art and include competitive binding assays and the like. Preferably, the antibodies bind p28<sub>TEV</sub> with high affinity.
In a specific embodiment, antibodies to p28<sub>TEV</sub> can bind to the V1 region of p28<sub>TCV</sub>. In some embodiments, the antibodies bind to one or more of the following peptides or variants thereof:

(20) NLNITKOTTNPTSSS (SEQ ID NO: 136),
(21) TKNTTNPTSSSWGMM (SEQ IDNO:137),
(22) TNPTSSSWGMEKGE (SEQ ID NO: 138),
(23) SSSWGMMEKGEIKNC (SEQ ID NO: 139) or,
(24) GMMEKGEIKNCFSFYI (SEQ BD NO: 140).

In other embodiments, the V1 polypeptide comprises an amino acid sequence selected from the group consisting of:

SSSWGMMEKGE (SEQ ID NO: 152),
SSSRGMVGGGE (SEQ ID NO: 153),
SSNWKEMDRGE (SEQ ID NO: 154), and
SSSGRMMIMEK (SEQ ID NO: 155).

In other embodiments, a V1 consensus sequence comprises a formula of contiguous amino acids comprising SSSX<sub>5</sub>MX<sub>7</sub>YX<sub>6</sub>G (SEQ IDNO: 156); wherein X4 is W, R, or G; X5 is G, K, or R; X7 is M, Vor I; X8 is E, G, D, or M; and X9 is K, G, R, or E.

In other embodiments, an isolated and purified V1 polypeptide comprises at least one V1 consensus sequence of a clade as shown in Figure 35. In some embodiments, an isolated V1 consensus sequence comprises for clade A:

CSNX<sub>5</sub>XSNTX<sub>9</sub>YX<sub>10</sub>TNTX<sub>11</sub>NX<sub>12</sub>,<sub>13</sub>TDGMREEKNC (SEQ ID NO: 160)

for clade B:

CTDLNNTX<sub>9</sub>X<sub>10</sub>NX<sub>11</sub>SSSGTMKMEKGEIKNC (SEQ ID NO: 161)

for clade C:

CTNVNENX<sub>8</sub>TX<sub>10</sub>NOX<sub>11</sub>GYX<sub>12</sub>NTYNSMX<sub>13</sub>2X<sub>14</sub>2X<sub>15</sub>2EIKNC (SEQ ID NO: 162)

for clade D:

CTDASRNX<sub>8</sub>TX<sub>10</sub>NOX<sub>11</sub>MEKGYMKNC (SEQ ID NO: 163)

for clade G:

CTNVNNX<sub>8</sub>TX<sub>10</sub>NOX<sub>11</sub>TX<sub>13</sub>NX<sub>14</sub>1<sub>15</sub>TVTX<sub>16</sub>EEEKNC (SEQ ID NO: 164)

for clade O:

CTNX<sub>8</sub>TX<sub>10</sub>NOX<sub>11</sub>TX<sub>13</sub>NX<sub>14</sub>1<sub>15</sub>TVTX<sub>16</sub>EEEKNC (SEQ ID NO: 165); wherein an X amino acid is any of the 20 naturally occurring amino acids.

Variants of these peptides have been described herein previously. These peptides can be used to generate or select antibodies specific for p28<sub>TEV</sub> polypeptide. In some embodiments, the immunogenic composition comprises one or more V1 sequences from a HIV isolate of a specific clade. In some embodiments, consensus sequences of the V1 region for each clade are included in the immunogenic composition.
In another embodiment, the antibodies have differential binding affinity for p28^{TEV} and Tat, Rev, or full-length Env. Preferably, antibodies with differential binding affinity have higher affinity for p28^{TEV} as compared to Tat, Rev, or full-length Env. Preferably, the binding affinity for p28^{TCV} is at least 100 fold greater than the binding affinity for Tat or Rev in order to allow for the differential detection of p28^{TEV}. In a further embodiment, the antibodies with differential binding affinity bind the VI region of p28^{TEV} at nanomolar concentrations and Tat, Rev, or full-length Env at micromolar concentrations. Screening assays and assays for determining the affinity of antibodies are known to those of skill in the art.

In another embodiment, the anti-p28^{THV} antibodies cross react with Rev or Tat. Antibodies that cross-react with p28^{TEV} and Tat or Rev are useful in method, for example, to screen for HIV infection. In a method for detecting p28^{TEV} in sample, p28^{TCV} is identified by molecular weight. The sample is immunoprecipitated with anti-p28^{TEV} antibody and the proteins in the immunoprecipitate are separated on a SDS gel and identified by molecular weight. p28^{TEV} has a molecular weight of 28,000 D and tat has a molecular weight of 16,000 or 14,000 D and rev has a molecular weight of 19,000 D. Alternatively, a combination of one or more anti-VI antibodies, anti-tat antibodies or anti-rev antibodies can be utilized to detect p28^{TEV} in samples.

Antibodies may be delivered intracellularly using a transport peptide such as described in Zhao et al., Apoptosis (2003) 8:631, or as described in U.S. 2005/0033033., Antibodies may also be expressed intracellularly in intrabodies. Intracellular antibodies to p28^{TEV} may inhibit viral replication. As p28^{TEV} is found in cell supernatants antagonist antibodies may also be employed as inhibitors.

H. Production of Antibodies
i. Polyclonal antibodies

Polyclonal antibodies to a p28^{TEV} polypeptide of the disclosure are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the polynucleotide encoding one or more p28^{TEV} polypeptides, or one or more p28^{TCV} polypeptide or fragments thereof, and optionally an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfo succinimid e ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl₂, or R\(^\text{N}=\text{C}=\text{NR}\), where R and R\(^1\) are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 µg or 5 µg of the protein or conjugate (for rabbits or
mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/2 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

In an alternative embodiment, the animals are immunized with a recombinant adenovirus vector expressing p28\textsuperscript{TEV} polypeptide of the disclosure followed by at least one booster immunization with p28\textsuperscript{TCV} polypeptides of the disclosure.

The polyclonal antibodies generated by the immunizations may undergo a screen for p28\textsuperscript{TEV} antagonist activity. Preferably, antibodies to the p28\textsuperscript{TEV} polypeptide decrease the infectivity of virus produced or inhibit viral levels. In an embodiment, antibodies that specifically bind a p28\textsuperscript{TBV} polypeptide comprising SEQ ID NO: 151 reduce or inhibit HIV viral levels. An antagonist antibody would be screened to determine if there was a decrease or inhibition of viral levels in infected cells expressing p28\textsuperscript{TBV}.

The polyclonal antibodies are also screened by enzyme-linked immunoabsorbent assay (ELISA) to characterize binding. The antigen panel includes all experimental immunogens. Animals with sera samples that test positive for binding to one or more experimental immunogens are candidates for use in monoclonal antibody production. The criteria for selection for monoclonal antibody production is based on a number of factors including, but not limited to, binding patterns against a panel of structured HIV immunogens.

Cross-competition experiments using other mapped Mabs, human sera and peptides can also be performed. Screening methods for identifying antibodies that bind to p28\textsuperscript{TEV}, for example, the V1 region, and do not bind to Tat and/or Rev are known to those of skill in the art and include competitive binding assays and the like. Screening assays and assays for selecting and identifying antibodies that have higher affinity for p28\textsuperscript{TCV} as compared to tat and/or rev are also known to those of skill in the art.

ii. Monoclonal antibodies

Monoclonal antibodies to a p28\textsuperscript{TEV} polypeptide of the disclosure may be made using the hybridoma method first described by Kohler et al., *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or macaque monkey, is immunized as hereinabove described to elicit lymphocytes
that produce or are capable of producing antibodies that will specifically bind to the p28\textsuperscript{TEV} polypeptide used for immunization. Alternatively, lymphocytes may be immunized \textit{in vitro}. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, \textit{Monoclonal Antibodies: Principles and Practice}, pp. 59-103 (Academic Press, 1986)). The hybridoma cells are then seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP\textsubscript{i} or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, \textit{J. Immunol}, 133:3001 (1984); Brodeur et al., \textit{Monoclonal Antibody Production Techniques and Applications}, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or enzyme-linked immunoabsorbent assay (ELISA).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, \textit{Monoclonal Antibodies: Principles and Practice}, pp. 59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown \textit{in vivo} as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.
The monoclonal antibodies are characterized for specificity of binding using assays as described previously. Antibodies can also be screened for antagonist activity as described previously.

iii) Human or Humanized antibodies

Humanized forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. Useful non-human antibodies are monoclonal antibodies that bind specifically to p28\textsuperscript{TEV}, for example, to the V1 region, and do not substantially crossreact with tat or rev antibodies. Useful non-human antibodies also include antibodies that inhibit or reduce viral levels. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or the donor antibody. These modifications may be made to improve antibody affinity or functional activity. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also the following review articles and references cited therein: Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23: 1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech* 5:428-433 (1994).

Human antibodies that specifically bind and/or antagonize p28\textsuperscript{TEV} transcriptional activity and/or inhibit viral infectivity can also be made using the transgenic mice available for this purpose or through use of phage display techniques.

In a specific embodiment, a humanized antibody is identified that specifically binds to the V1 region of p28\textsuperscript{TEV}. Preferably, the humanized antibody binds to one or more of peptides corresponding to peptides 20-24 of the VI region p28\textsuperscript{TEV} as described herein. In a specific embodiment, a VI peptide is selected from the group consisting of:

\begin{itemize}
  \item[(20)] NLN\textsuperscript{\textit{T}}KNTTNPTSSS (SEQ BDNO:136),
  \item[(21)] TELNTNPTSSSWGMM (SEQ ID NO: 137),
  \item[(22)] TNPTSSSWGMMKEKGE (SEQ ID NO: 138),
  \item[(23)] SSSWGMMEKGEKNC (SEQ ID NO: 139), and
  \item[(24)] GMMEKGEKNCFSFYI (SEQ ID NO: 140).
\end{itemize}
In other embodiments, the V1 polypeptide comprises an amino acid sequence selected from
the group consisting of:

SSSWGGMMEKGE (SEQ ED NO: 152),
SSRGMVGGGE (SEQ ID NO: 153),
SSNWKEMDRGE (SEQ ID NO: 154), and
SSSGRIMMEKG (SEQ ID NO: 155).

In other embodiments, a V1 consensus sequence comprises a formula of contiguous amino
acids comprising SSSX₄S₃MX₇X₈X₉GE (SEQ DDNO: 156); wherein X₄ is W, R, or G; X₅ is
G, K, or R; X₇ is M, V, or I; X₈ is E, G, D, or M; and X₉ is KₚGₚR, or E.

These peptides can be used to generate or select antibodies specific for p28^{TEV} polypeptide.
In some embodiments, the immunogenic composition comprises one or more V1 consensus
sequences from a HIV isolate of a specific clade. In some embodiments, consensus
sequences of the V1 region for each V1 clade are included in the immunogenic composition.

In other embodiments, an isolated and purified V1 polypeptide comprises at least
one V1 consensus sequence of a clade as shown in Figure 35. In some embodiments, an
isolated V1 consensus sequence comprises for clade A:

CSNX₄X₅NNTX₉X₆NTNX₁₅TDGMREEKNC (SEQ ID NO: 160)

for clade B:

CTDLNNTNX₉X₁₀TSSSGGTMEKGEIKNC (SEQ DD NO: 161)

for clade C:

CTNVINX₅TXIoX₁₀NTNX₁₅TDGMREEKNC (SEQ DD NO: 162)

for clade D:

CTDAOSNX₉TX₁₀NNTNGPX₁₅MEKGMKNC (SEQ H NO: 163)

for clade G:

CT₅VNX₄X₅X₆X₇X₈X₉XₐX₁₀X₁₁TVTX₂₀EEEKNC (SEQ IID NO: 164)

for clade O:

CTNX₄X₅GTTX₈X₉X₁₀X₁₁X₁₂X₁₃X₁₄X₁₅X₁₆X₇SENLMKQC (SEQ ID NO: 165);

wherein an X amino acid is any of the 20 naturally occurring amino acids.

iv. Antibody conjugates

The antibodies specific for a p28^{TEV} polypeptide or fragment thereof can be
combined with heterologous moieties to provide a detectable label or for targeted delivery
of an inhibitory agent.

Detectable labels include radionuclides, biotin, dyes, enzymes, and fluorescent
molecules.

Inhibitory agents include cytotoxic agents such as toxins, and siRNA molecules.
SiRNA molecules are described herein specific for a p28^{TEV} polypeptide or fragment.
thereof. Other siRNA molecules can be constructed that are specific for other HIV proteins such as Tat, Rev or reverse transcriptase.

I. Uses and Methods

The p28\textsubscript{TEV} polypeptides and/or polynucleotides as described herein can be utilized in immunogenic compositions, diagnostic assays, to develop monoclonal antibodies, and as potential targets for other inhibitory agents.

In one aspect of the disclosure, a method is provided for immunizing a mammal with an immunogenic composition as described herein. A method for inhibiting HIV viral levels comprises administering at least one isolated nucleic acid encoding a p28\textsubscript{TEV} polypeptide or fragment thereof. In some embodiments, the fragment of the p28\textsubscript{TEV} polypeptide includes all or a portion of the V1 region. In some embodiments, the immunogenic composition comprises an immunomodulator or adjuvant. In some embodiments, the immunogenic composition may comprise at least one isolated and/or purified p28\textsubscript{TEV} polypeptide or fragment thereof.

The p28\textsubscript{TEV} polypeptides are useful to develop antibodies to the p28\textsubscript{TEV} polypeptides. Methods for developing antibodies have been described herein. Antibodies may be useful in diagnostic assays for detecting the presence of HIV in a biological sample. One aspect of the disclosure provides a method for screening for HIV, comprising contacting a biological sample with at least one anti-p28\textsubscript{TEV} polypeptide antibody and assaying the biological sample for anti-p28\textsubscript{TEV} antibody binding of the protein. Antibodies to V1, tat and rev can be used in combination to detect HIV infection. Antibodies are also useful to purify the p28\textsubscript{TEV} polypeptides or provide for targeted delivery of a cytotoxic agent.

p28\textsubscript{TCV} polypeptides may be useful in diagnostic or prognostic assays. The presence or absence of an antibody to a p28\textsubscript{TEV} polypeptide in a biological sample can be determined using standard methods. Alternatively, presence or absence of p28\textsubscript{TEV} polypeptide in a biological sample can be determined using PCR primers specific for nucleic acids encoding p28\textsubscript{TEV} polypeptides to amplify any HIV-I DNA that may be present in the sample.

The disclosure also provides methods for screening for agents that may inhibit or antagonize p28\textsubscript{TEV} polypeptides. The p28\textsubscript{TEV} polypeptide identified herein may have a role in regulating HTLV infectivity. Novel therapeutics may be identified that can inhibit the expression or activity of these p28\textsubscript{TEV} polypeptides. One aspect of the disclosure provides a method for screening for agents that inhibit a p28\textsubscript{TEV} polypeptide comprising expressing a p28\textsubscript{TEV} polypeptide in a cell or liposome; contacting the cell with an agent and determining whether the agent inhibits the transcriptional activity and/or effect of p28\textsubscript{TEV} polypeptide on
HIV viral levels. The effect of p28\textsuperscript{TEV} polypeptide or inhibition thereof may be quantified by transactivation measured in a Luc assay.

Potential agents that can act to inhibit the effect of p28\textsuperscript{TEV} on HTV-I production include antagonist antibodies, antisense RNA specific for p28\textsuperscript{TEV}, small interfering RNA (siRNA) approaches, microRNA or methods to modulate pre-mRNA splice site choice. Antagonist antibodies can be prepared and screened as described previously.

p2g\textsuperscript{TEV} antagonists or immunogenic compositions of the disclosure can be used in combination with one or more antiviral compositions to treat patients infected with HIV. Antagonists of p28\textsuperscript{TEV} polynucleotides or polypeptides or immunogenic compositions can be administered prior, concurrent, or subsequent to administration of the one or more antiviral compositions. The antiviral compositions can be nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, or attachment inhibitors. Examples of nucleoside reverse transcriptase inhibitors include, but are not limited to, AZT,ddl (didanosine), ddC (zalcitabine), d4T ( stavudine), Abacavir, Tenofovir, Combivir, Trizivir, Emtricitabine, Epzicom, and Truvada. Examples of nonnucleoside inhibitors include, but are not limited to, Nevirapine, Delavirdine, and Efavirenz. Examples of protease inhibitors include, but are not limited to, Saquinavir, Indinavir, Ritonavir, Nelfinavir, Amprenavir, Lopinavir, Atazanavir, and Fosamprenavir. Examples of fusion inhibitors include, but are not limited to, Enfuvirtide.

In an embodiment, one or more p28\textsuperscript{TEV} antagonists or immunogenic compositions of the disclosure is administered in combination with one or more antiviral compositions to a patient infected with HIV-I. The patient can have a viral load that is "undetectable" by a viral load test. Methods for determining viral load are known. The p28\textsuperscript{TEV} antagonists or immunogenic compositions can be administered prior, concurrent, or subsequent to administration of the one or more antiviral drugs.

p28\textsuperscript{TEV} antagonists or immunogenic compositions of the disclosure are useful for decreasing the probability of developing HTV that are resistant to antiviral compositions. Guidelines for treating HIV and preventing development of resistance to therapeutic compositions with combination therapies are known. See, for example, the U.S. Department of Health and Human Services guidelines for medical management of HTV in adult patients, adolescent patients, or pediatric patients at www-aidsinfo-nih-gov/guidelines.

In an embodiment, two or more p28\textsuperscript{TEV} antagonists of the disclosure, preferably three or more p28\textsuperscript{TEV} antagonists of the disclosure, are administered to a patient infected with HIV-I to decrease the probability of the patient developing virus that is resistant to a p28\textsuperscript{TEV} antagonist of the disclosure. In an embodiment, one or more p28\textsuperscript{TEV} antagonists or immunogenic compositions of the disclosure are administered in combination with one or more, preferably two or more, preferably three or more antiviral compositions to a patient...
infected with HIV-I to decrease the probability of the patient developing virus that is resistant to any of the antiviral compositions or antagonists of the disclosure in the combination therapy. The antiviral compositions can be a combination of nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, and attachment inhibitors. The p28\textsuperscript{TEV} antagonists or immunogenic compositions can be administered prior, concurrent, or subsequent to administration of the one or more antiviral compositions.

The following examples are provided for illustrative purposes only, and are in no way intended to limit the scope of the present disclosure.

**EXAMPLES**

**EXAMPLE 1**

HIV-I p28\textsuperscript{TEV} is Expressed in the Nucleolus and Cytoplasm of a Cell

Previous reports concerning expression of p28\textsuperscript{TEV} in infected cells indicated that p28\textsuperscript{TEV} was only found in the nucleolus. Benko et al. cited supra. The HTLV-I protein p30\textsuperscript{1} is thought to restrict viral replication and contribute to a latent state in HTLV-I infected cells. p30\textsuperscript{II} is also found only in the nucleolus of infected cells. We investigated the location of expression of p28\textsuperscript{TEV} in HeLa cells transfected with cDNA encoding p28\textsuperscript{TEV}.

**Materials and Methods**

DNA constructs: A cDNA construct encoding p28\textsuperscript{TEV} was constructed as described in Benko et al. cited supra. The cDNA encoding p28\textsuperscript{TCV} was fused to at its C terminus to a sequence encoding green fluorescent protein by cloning into the EcoRI restriction site in pGFP-C3 vector (Promega Corp, Madison, WI). The p30\textsuperscript{II} cDNA was subcloned into EGPN3 at the HindU-EcoRI site so that the green fluorescent protein was fused at the amino terminal end of the protein.

Transfections: Transfections were performed using 5 micrograms of DNA into HeLa cells using electrophoresis.

Detection of Expression: Expression of p28\textsuperscript{TEV} and p30\textsuperscript{H} was detected in cells using confocal microscopy.

**Results**

As shown in Figure 1, p28\textsuperscript{TCV} is not only in the nucleolus, as previously reported by Benko et al., 1990, *J. Virol.*, 64:2505-2518, but also in the cytoplasm. In contrast, HTLV-I p30\textsuperscript{II} is located in the nucleolus and nucleoplasm. Like Tat, p28\textsuperscript{TEV} is found in the supernatant and may be capable of uptake by neighboring cells (data not shown).
EXAMPLE 2

Overexpression of HIV-1 p28<sup>TEV</sup> Inhibits Viral Production

As described previously, HIV-I infection results in production of multiply spliced mRNAs that encode transcriptional regulatory proteins, Tat and Rev. The function of p28<sup>TEV</sup> in infected cells has not been described. The p28<sup>TEV</sup> polypeptide retains both Tat and Rev functions. We investigated the function of p28<sup>TEV</sup> by coexpressing it with HXB2 provirus in 293T cells.

Construction of DNA Plasmids

To assess the activity of p28<sup>TEV</sup> polypeptide, we expressed p28<sup>TEV</sup> in trans simultaneously with the HXB2 provirus. The infectious HTV-I proviral clone HXB2 was used in all experiments and has been previously described (Fisher et al., 1985, Nature, 316:262-265). The HXB2 clone was obtained from Fisher et al.

The p28<sup>TEV</sup> construct pNL1.4.6D.7 was constructed by inserting a cDNA encoding p28<sup>TEV</sup> into a cDNA expression plasmid. Benko et al., 1990, J. Virol, 64:2505-2518; Schwartz et al., 1990, J. Virol, 64:2519-2529. The BssHII-to-BamHI fragment of the cDNA expression plasmid was ligated with the 7.1 kilobase-pair BssHII-to-BamHI fragment of pNL43, which provided the HTV-ILTR promoter and polyadenylation signal. Benko et al., 1990, J. Virol, 64:2505-2518.

p28<sup>TEV</sup> polypeptide activity was assessed with the HIV-I-Luc reporter gene. The HTV-I-Luc reporter construct was prepared as described by M. R. Smith & W. C. Greene, 1990, Genes Dev., 4:1875-1885.

We performed all experiments adding RT-LK as an internal control for efficiency of transfection. The RT-LK vector was obtained form Promega Corp.

Luc Assay

HIV p28<sup>TEV</sup> retains the Tat activation/elongation domain. Therefore, p28<sup>TEV</sup> polypeptide activity was assessed with the HTV-I-Luc reporter gene. 293T human kidney cells were maintained in Dulbecco’s Modified Eagle Medium supplemented with 5% Fetal Bovine Serum, 1% L-glutamine, and 1% Penicillin-Streptomycin (Invitrogen, Grand Island, NY). The 293T cells were transformed using Effectene (Qiagen, Valencia, CA). 0.5, 1, 5, and 10 micrograms of DNA were transfected. Transfection efficiencies were normalized using RL-TK.

The infectivity assay is expressed as luciferase units as the virus is applied to HeLa cells expressing on the cell surface the HTV-I receptors CCR5 and CD4 and CXCR4 and carry an HIV-I LTR luciferase construct that can be activated following viral infection and production of the viral transcriptactivator, Tat.
Viral Production Assay

Viral production was assessed 24 hours after transfection by quantifying the amount of HIV-1 p24 in the supernatant of cell cultures by enzyme-linked immunosorbent assay (ELISA) or intracellular expression of p24 by Western blotting.

ELISA was used to measure p24 in the supernatant. p24 was measured 24 hrs after transfection with the Retro-Tek HIV-1 p24 antigen ELISA kit (ZeptoMetrix Corp., Buffalo, NY). Ninety-six-well Nunc Maxisorp microliter plates (Fisher Scientific Co., Pittsburgh, Pa.) were coated at 4°C overnight with monoclonal antibody specific for p24 core protein. The plates were washed 6 times with 1X plate wash buffer. Two hundred microliters of supernatant diluted with 25 microliters of lysing buffer was added to each well and the plate was incubated for 2 h at 37°C. The plate was washed as described above and then incubated with HIV-I p24 detector antibody for 1 h. The plates were washed four times with IX plate wash buffer, developed with streptavidin-peroxidase working solution and substrate working solution for 30 min, and the absorbance was measured at 450 nm with a Victor multilabel counter spectrophotometer (PerkinElmer, Boston, MA).

Western blots were performed as previously described. Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989. Transfected cells were lysed with 2% deoxycholic acid, 2% SDS, 2% Triton X, 0.3M NaCL, and 1M Tris pH 7.4 (RIPA buffer) with 1 ug/ml aprotinin, ImM DTT, and ImM PMSF. Forty to fifty micrograms total protein was transferred to nitrocellulose membrane (BioScience, Keene, NH) in IX Tris/Glycine buffer (Bio-Rad, Hercules, CA) at 85V for 2 hrs at 4°C. The membrane was blocked with 5% milk in 0.1M NaCl, 0.01M Tris-HCL pH 7.5 and 0.001M EDTA (TNE buffer) for 30 min at RT. The membrane was rinsed twice and incubated with a monoclonal antibody to HIV-I p24 (Advanced BioScience Laboratories, Inc., Rockville, MD) in TNE buffer at 1:250 o/n at 4°C. The membrane was washed for 1 hr in TNE buffer + 5% Triton X-100 with vigorous shaking and incubated with HRP-linked goat anti-mouse IgG (Santa Cruz Biotechnology, (Santa Cruz, CA) in TNE buffer at 1:8000 for 1 hr. The membrane was rinsed and washed as described above and visualized using a commercial ECL detection kit (Amersham Biosciences, Buckinghamshire, England).

Results

As shown in Figure 2A, the efficiency of transfection was comparable in all experiments. The results also show that transfecting cells with increasing amounts of p28TEV increased Luc from the HIV-I-Luc reporter gene (Figure 2B), indicating that expression of p28TEV resulted in increasing tat activity. Despite an increase in Tat activity of the p28TEV polypeptide (Figure 2B), viral production as measured by HIV-I p24 in the supernatant was surprisingly decreased (Figure 2C). We also observed a decrease in intracellular production of p24 and an increase in tev by Western blot (Figure 2D) using
antibodies directed to Rev. The size of Tev was differentiated by that of Rev using molecular weight markers.

To rule out the possibility that exogenous HIV-I-Luc construct was competing for the transactivation of Tat produced by the HXB2 provirus, the experiments were repeated in the absence of the HTV-I-Luc construct. In the absence of HIV-I-Luc construct, we observed a dose-dependent decrease in p24 production as the amount of p28$^{TEV}$ increased (Figures 3A and 3B).

When HXB2 p28$^{TEV}$ is coexpressed in trans with the provirus HXB2, viral production as measured by p24 production is decreased. The overexpression of p28$^{TCV}$ in combination with HXB2 HIV isolate may be acting to inhibit viral production by competing with Rev for RNA binding.

**EXAMPLE 3**

**Loss of expression of p28$^{TEV}$**

We have investigated Tev's function in detail by generating three HIV-I molecular clones in which the acceptor splice site within the Env exon of p28$^{TEV}$ was mutated without altering the amino acid sequence of the Env V1 region of the protein. This mutation results in the loss of expression p28$^{TEV}$ from the clones.

**Preparation of Plasmids**

Each of pHXB2, pNL and 89.6 were altered at the acceptor splice site within the ENV exon without altering the ENV amino acid sequence. (See Figures 9, 26-33.) The acceptor splice site has a sequence of AGTTTA (SEQ ID NO: 13) which is changed to the sequence TCACTG (SEQ ID NO: 12) by amplifying the sequences using a PCR primer that changes the splice acceptor site. The sequence of the primer is

5'-CTC TGT GTT TCA CTG AAG TGC ACT-3' (SEQBDNO:11).

The change to the acceptor splice site prevents expression of p28$^{TEV}$. The altered clones are designated pHXB2$^{Atev}$, pNL4-3$^{Atev}$, and SHTV 89,6$^{Atev}$. SHIV89.6 can be obtained from the NIH AIDS Research and Reagent Program. The clone pME was used as a control and was used previously [Nicot et al. "HTLV-I-encoded p30 is a post-transcriptional negative regulator of viral replication", Nat Med., 10(2): 197-201 (2004)].

**Infectivity Assay**

Each of the clones were used to infect TZM or transfect 293T cell cultures. TZM cells are available from the NIH AIDS Research and Reagent program. TZM cells carry luciferase and β galactosidase genes under control HIV-I promoter. Viral production was measured by detecting p24 production in the supernatant at 24 and 48 hours. The cell cultures were lysed at 24 and 48 hours and run on SDS PAGE gels. The gels were stained with Coomassie blue.
Results

The results are shown in Figures 10 and 11. The results in Figure 10A and B show that the pHXB2 Δtev clone results in a decrease of viral infectivity at 24 and 48 hours of cell culture. When cell lysates were run on SDS PAGE (Figures 10C and 10D), the HXB2 clone lacking p28\textsuperscript{TEV} expression had increased expression of Rev and decreased expression of p55 and p42.

293T cell cultures were each transfected with pME, pHXB2, pHXB2Δtev, pNL4-3, pNL4-3 Δtec, and SHIV89.6. Viral production in the cell cultures were measured at 24 and/or 48 hours as shown in Figure 10A, 10B and 11A. Transfection of 293T cells with pHXB2 Δtev resulted in decreased viral productivity as measured by p24 production. However, pNL4-3 Δtev and SHTV89.6tev did not show any decrease in viral production.

The results of the luciferase assay and reserve transcriptase assay show that the supernatant of pHXB2 Δtev in TZM cell cultures was less infectious. The expression of luciferase and reverse transcriptase was significantly decreased in the HBX2 clone lacking tev expression. In contrast, as shown in figure 11D and 11E, cell cultures infected with pNL4-3 Δtev and SHIV89.6 Δtev did have comparable activation of Tat-driven luciferase and had equivalent amount of reverse transcriptase activity.

When cell lysates of the cell cultures were analyzed using SDS PAGE, the expression of gpl60 was comparable in all cell cultures, except in cells infected with pHXB2 Δtev (see Figures 10C, 10D and 11B). In the cells infected with pHXB2 Δtev, expression of gpl60, p55, p42, and p24 were decreased, while rev was greatly increased.

The difference between pHXB2 Δtev, pNL4-3 Δdev and SHTV89.6 Δtev is that cell cultures infected with pHXB2 Δtev had an increase in Rev expression. The increase in Rev expression may result in inhibition of translation and decrease in production of viral proteins such as reverse transcriptase, gpl60 and p24.

The effect of increasing amounts of Rev on translation was determined by adding an increasing amount of cDNA encoding Rev to 293 cells transfected with pNL4-3 Δtev. The results are shown in Figure 11C. When increasing amounts of Rev were added to the cells, p24 productivity decreased.

EXAMPLE 4

DNA Plasmids Expressing p28\textsuperscript{TEV}

We generated three DNA plasmids expressing Tev from the CCR5 users SF1 62, BaL, and the CXCR4 user simian-human immunodeficiency virus (SHIV)-89.6P and demonstrated their expression in 293T cells (Fig. 12B) and compared their protein products to the pHXB2 Tev obtained by Benko et al. The DNA plasmid constructs were produced as
described below. The 89.6P protein was expressed in E. coli and purified (Fig. 12C) using conventional techniques.

**Plasmid preparation**

Synthetic genes encoding p28\textsuperscript{tev} from each of BaL, SFI 62, HXB2, and 89.6 were prepared using synthetic oligonucleotides and codons were optimized for human and E. coli cells. The sequences of the genes are provided in Figures 26-33. Published sequences for each isolate includes M68893, M65025, U39362, and M37898. The genes were cloned into a vector pPCR-Script-Amp (Stratagene, CA) using KpnI and SacI restriction sites as shown in Figure 34A-D. The plasmid DNA was purified and concentration determined by UV spectroscopy. The final construct was verified by resequencing as shown in figures 26-33.

**DNA Vaccine Vectors**

DNA vaccine vectors were constructed for Ba-L, SF162, and 89.6\textsuperscript{Tev}: pCMV Ba-L Tev, pCMV SFI 62 Tev, and pCMV 89.6P Tev, respectively. Briefly, for each tev construct, a SacI/EcoRI DNA fragment encoding the tev gene was ligated into a mammalian expression vector derived from pVR1332 (Vical, San Diego, CA). Plasmids are kanamycin resistant, and the expression of Tev is under control of the CMV immediate-early promoter. Plasmid DNA was produced at Althea Technologies (San Diego, CA) at the concentration of 1mg/ml in sterile water. DNA was found to be >95% circular and predominantly supercoiled by gel electrophoresis, with endotoxin levels below 1.5 EU/mg DNA.

Tev vaccine vectors were tested and found to be functional by radioimmunoprecipitation assay (RIPA) of transiently transfected BEK 293 cells. (Figure 12 B) Transfections were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) using the manufacturer’s recommended protocol. Wells of a twenty-four-well tissue culture plate were seeded with 2x10\textsuperscript{5} HEK 293 cells in 500 µl DMEM (Quality Biological, Gaithersburg, MD) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS; Hyclone, Logan, UT) and 2 mM L-glutamine (Quality Biological). Cultures were incubated for 18 h at 37°C with 5% CO\textsubscript{2}. For each plasmid, 2.5 µl of Lipofectamine 2000 reagent was mixed with 47.5 µl Opti-MEM Reduced Serum Media (Invitrogen) and set at room temperature for 10 min. For each plasmid, 1 µl (1 µg) DNA was mixed with 49 µl Opti-MEM Reduced Serum Media, and set at room temperature for 5 min. DNA and Lipofectamine 2000 solutions were combined and set at room temperature for 20 min, then added to the 18 h HEK 293 cultures. Cultures were further incubated at 37°C with 5% CO\textsubscript{2} for 23 h.

At 23 h post-transfection, transfected cells and negative control cells (neat HEK 293) were washed once with 1 ml D-PBS (Quality Biological) and overlaid with 500 µl L-
methionine and L-cystine free DMEM (Invitrogen) supplemented with 2% heat-inactivated
diazyed FBS (Invitrogen), 2 mM L-glutamine (Quality Biological), 1 mM sodium pyruvate
(Quality Biological) and 30 mg/ml L-methionine (Sigma, St. Louis, MO). Cells were
labeled with 50 µCi ^35S Cysteine (Perkin Elmer, Shelton, CT) and incubated for 16 h at
37°C with 5% CO_2 for 23 h. Media was removed and cells were lysed in PBS-TD (PBS
with 0.5 % Triton-X-100 and 1 % deoxycholic acid) containing 1 µl/ml benzonase nuclease
(EMD Biosciences, Madison, WI) and 1 µl/ml Protease Inhibitor Cocktail for Mammalian
Cells (Sigma) for 10 min at room temperature. Lysates were clarified by centrifugation at
10,000 X g for 5 min. Lysates were precleared by shaking with 20 µl protein A sepharose
(PAS; Amersham Biosciences, Piscataway, NJ) and 5 µl normal rabbit serum at room
temperature for 1 h. Ten percent glycerol was added to the precleared lysates, and lysates
were immunoprecipitated with 20 µl PAS and 5 µl rabbit anti-HIV-1_MNRev serum
(Advanced BioScience Laboratories, Kensington, MD) or 5 µl rabbit anti-HrV-InIB Tat
serum (Advanced BioScience Laboratories) for 2.5 h at room temperature. Precipitated
samples were washed three times with 1 ml PBS-TD and boiled for 2 min in 100 µl
reducing SDS-PAGE sample buffer. Precipitated proteins were separated on a 10% SDS-
PAGE, gels were soaked in destaining solution (40% methanol and 7 % acetic acid) for 45
min, then soaked in Amplify (Amersham Biosciences) for 30 min. as shown in figure 12B.
Gels were dried to filter paper and exposed to x-ray film (BioMax MR, Eastman Kodak,
Rochester, NY) and developed after 20 h.

**Purification of D2S^sv**

The 89.6P Tev protein was produced in E. coli and purified using immunoaffinity
chromatography. The 89.6P tev gene was amplified from the pPCR-Script Amp SK vector
containing the synthetic 89.6P tev gene using the following primers: 89.6P Tev 5’ Nde
(acttagcatatggaacctgtgaaccctagctgga (SEQ ID NO:300)) containing the Ndel site and 89.6P
Tev 3’ Xho (ctaatgctcagttacttctggctccaggttc (SEQ ID NO:301)) containing the Xhol site.
The Ndel/Xhol DNA fragment encoding the tev gene was ligated into the bacterial
expression plasmid pET26 (EMD Biosciences, Madison, WI). The resulting pET26 89.6P
Tev plasmid is kanamycin resistant, and the expression of Tev is under control of the IPTG-
inducible T7 lac promoter. BL210DE3 competent cells (EMD Biosciences) were
transformed with pET26 89.6P Tev, and the Tev sequence of clones were confirmed by
DNA sequence analysis.

One liter culture of the 89.6P Tev E. coli cell line was grown at 37°C to 0.9
OD_{600}_{nm} cooled to 22°C and induced with 1 mM IPTG for 6 h. Cells were harvested by
centrifugation and lysed in 40 ml of a PBS-based buffer containing 0.5 M sodium chloride,
10 mM ascorbic acid, 50 mM mannitol, 2.5 % glycerol, 0.5 % Triton-X-100, protease
inhibitor cocktail, 50 µg/ml lysozyme, 1 µl/ml Benzonase nuclease (EMD Biosciences), and 5 mM DTT. Lysates were sonicated and clarified by centrifugation. Tev was purified from the lysate on mouse monoclonal antibody to HIV-I Tat sepharose, using 100 mM sodium carbonate with 5 mM DTT for elution. The eluate was buffered with phosphate buffer and the pH was adjusted to 7.4 with hydrochloric acid. Protein was passed through a goat anti-mouse IgG sepharose column to remove any mouse antibody that may have leached off of the immunoaffinity column. Endotoxin was reduced to about 400 EU/mg protein using Detoxigel (Pierce Biotechnology, Rockford, IL). The final Tev product runs as a 28 kDa protein on SDS-PAGE with a purity of about 90%. A Western blot, 89.6P Tev reacts well with rabbit anti-sera to HIV-I gp120, HIV-I Tat, and HIV-I Rev, demonstrating that the Tat, Env, and Rev components that compose Tev are present and immunologically recognized.

Results

We have constructed cDNAs that expressed the Tev protein from the HXB2, 89.6P, SF162, or Bal HIV-I isolates that include the first exon of Tat, V1, and the last exon of Rev, as such a protein is naturally encoded by the pHXB2 isolate (Fig. 12A). Figure 12A shows a comparison of the amino acid sequences of p28 tev from the different isolates. All these cDNAs expressed the expected 28 kDa protein upon transfection into 293T cells. (Fig. 12B) As shown in Figure 12, the DNA plasmids SF1 62, Bal, pHxb2, and 89.6P each express p28 TEV. DNA plasmid 89.6P was introduced into E. coli and p28TEV was produced and isolated as described above. (Figure 12C)

EXAMPLE 5

Immunization Protocols

The Bal, SF162, 89.6P plasmid DNAs were used to immunize Mamu-A*01 + macaques and the purified protein from 89.6P used to boost antibodies and other virus-specific immune responses as indicated in Fig. 12D. Animals were immunized with DNA plasmids encoding p28 TEV at 0, 3, and 8 months. Booster injections of p28 TEV polypeptide 89.6 in combination with an immunomodulator CpG were administered at 12 and 18 months. At 22 weeks, the animals were challenged with SHTV89.6P.

Plasma antibody titers for Tev, Tat, HTV MN, Rev, and Env V1 peptide pool were determined in the immunized animals at 5, 10, 14, 19, 22, 27 and 30 weeks. Viral load in the animals was measured post challenge at 22, 24, 26, 28, 30, 32, 34, 35, 36, 38, 40, 42, 44 and 46 weeks. CD3/4 cell counts were also taken post challenge at the same time periods.

Antibody titers were determined in ELISA or ELISPOT assays. Control animals received CpG rejections at 12 and 18 months.
Viral Load Assay

A number of different assays are available for determining viral load from biological samples, such as serum or tissues. Assays such as the Amplicore assay available from Roche Diagnostics is a PCR-based assay that can detect SHIV RNA. The assay utilized was the NASBA assay as described by Romano et al., *Immunological Investigations*, 26:15-28 (1997). NASBA assay kits for HIV-I are available as NucleoSens Easy Q® HIV-I, V1.1 (BioMerieux, France). A representative NASBA assay is described below.

Briefly, one volume of tissue or serum is added to 9 volumes of lysis buffer (5.25 MGuSCN, 50 mM Tris, ph 7.2, 20 mM EDTA, 1.3% Triton X-100). Next 50 µl of acid treated silica is added to the lysate and is pelleted by centrifugation and washed. The pellet is washed twice with ethanol and once with acetone. The acetone is evaporated from the silica and the nucleic acids are eluted from the silica into water.

The amplification reaction includes 5 µl nucleic acid extract, 10 µl of 80 mM Tris, ph 8.3, 24 mM MgCl₂, 140 mM KCl, 10 mM DTT, 2.0 mM each dNTP, 4mM each NTP, 30% DMSO, 0.4 µM Primer 1 and 0.4 µM primer 2. HIV primers are described in Van Gemen, *J. Virol. Meth.*, 49: 157 (1994) and herein. This mix is heated to 65°C for 5 minutes and then cooled to 41°C. Once cooled, 5 µl of enzyme mix (6.4 units/µl T7 RNA polymerase, 1.3 units/µl AMU-RT, 0.02 units, 1 µl RNase H, 0.42 µg/µl BSA) is added and incubated for 90 minutes at 41°C. The reaction product is then characterized by hybridization analysis.

Probes can be labeled with a detectable label and then used in southern blots, enzyme linked gel assays, or electrochemoluminescence system to detect the reaction products.

CD3/CD4 Cell Count

White blood cell counts in vaccinated and control animals after challenge with SHIV89.6 were conducted at two week intervals from 22 weeks to 44 weeks. CD3/CD4⁺ positive cells were quantitated using flow cytometry or ELISPOT assays.

Identification of effector CD4⁺ and CD8⁺ T cells

Fresh peripheral blood mononuclear cells (PBMCs) were isolated using LSM (Lymphocyte Separation Medium) (Cappel, Aurora, Ohio) density centrifugation. In some instances PBMC were frozen (90% fetal calf serum (FSC) 10% DMSO) until use.

10⁶ PBMC were incubated with 1µg/ml each of antibodies CD28 and anti-CD49d and 1µg/ml of Gag pool peptide in a 1-ml volume. Conjugated antibodies to the granular membrane proteins CD107a were kindly supplied by M.R. Berts (Laboratory of Immunology, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland) and were added to the cells.
prior to stimulation. A negative control (anti-CD28, anti-CD49d), and positive control (SEB, 1 µg/ml) were included to control for spontaneous, and activated production of cytokines and/or expression of CD107a. The cultures were incubated for 1 h at 37°C in 5% CO₂, followed by an additional 4 hours in the presence of the secretion inhibitor monensin (BD Pharmingen) and Brefeldin A (Sigma). After stimulation, PBMC were washed, and surface stained (anti-CD4, BD Pharmingen); anti-CD8j3, Immunotech), washed again, and after then permeabilized. After permeabilization, the cells were washed and stained with antibodies specific for intracellular markers (IFN-γ and TNF-α monoclonal antibodies, BD Pharmingen). The cells were washed a final time and resuspended in 1% paraformaldehyde (Electron Microscopy Systems, Fort Washington, Pennsylvania) in PBS.

Four-parameter flow cytometry analysis was performed using a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems). List mode data files were analyzed using FlowJo software (Tree Star, San Carlos, California). In all cases at least 100,000 live events were collected for analysis.

**ELISPOT Assays**

ELISPOT assay was performed with the monkey-specific IFN-γ Millipore kit (Billerica, Massachusetts), following the manufacturer's specifications. Briefly, after coating with anti-IFN-γ monoclonal antibody, plates were incubated overnight at 4°C and washed and wells were covered with 5% human serum in PBS at room temperature for 2 h. After washing, PBMCs (2x10^5/50 µl) were plated per each well in RPMI-1640 containing 5% heat-inactivated human serum and stimulated in triplicate with an Env peptide pool at 1 µg/ml. Concanavalin A (5 µg/ml) was used as a positive control. After 18 h incubation at 37°C in a 5% CO₂ atmosphere, plates were washed and coated with filtered rabbit polyclonal anti-IFN-γ biotinylated detector antibody for 2 h. Plates were developed with chromogen substrate activated by alkaline phosphatase in streptavidin-AP conjugate and SFCs counted on an ImmunoSpot Analyzer (CTL LLC, Cleveland, Ohio). In this assay, results were considered positive at more than 25 spot-forming cells/10⁶ cells, based on background levels obtained with cells from Mamu-A*01-negative animals using Mamu-A*01-restricted CTLs) epitopes or stimulation with irrelevant peptides.

**ELISA Assay**

Briefly, ELISA plates were coated overnight at 4°C with 100 µl/well 50 mM sodium bicarbonate buffer (pH 9.6) containing 100 ng/well BSA plus: 50 ng/well for rHIV-iHXB2 Tat, THIV-Igα6p TeV, THIV-EnvRev proteins, apool of25 ng/well each recombinant gp140: Ba-Lα, SF162 P3, and 89.6F gp140, or a pool of 50 ng/well each Env V1 peptide: Ba-L, SF162, and 89.6P V1 peptides. Plates were blocked at room temperature for 2 h with 200 µl/well 5% sucrose, 1.25% nonfat dry milk and 2.5% BSA. Serum samples were
diluted in Dilsim π (BioMerieux, Durham, NC), 100 µl/well were added to plates, in
duplicate, and incubated for 1 h at 37°C. Plates were washed four times with PBS + 0.5%
tween 20. Plates were incubated at 37°C with 100µl/well goat anti-human IgG HRP
conjugate diluted in PBS plus 20% NGS and 0.1% Triton-X-100 for 1 h. Plates were
washed four times in PBS plus 0.5% tween 20. Plates were incubated for 30 min at room
temperature with 100 µl/well 1MB substrate, and stopped with 100 µl/well 2 N sulfuric
acid. Absorbance was read at 450 nm. End point titers were calculated by determining the
dilution at which a sample's absorbance value equals two times the absorbance of the pre-
bleed sera at 1:50 dilution.

**Pepscans**

A library of forty-eight peptides spanning the entire length of the HPZ-I\textsubscript{89-69} Tev
protein was synthesized. Peptides are fifteen residues in length and overlapped the flanking
peptides by eleven residues. Peptides 1..18 are derived from the first exon of Tat; peptides
16..28 are derived from the Env V1 region; peptides 26..48 are derived from the second
exon of Rev. ELISA plates were coated at 4°C overnight with 100 µl/well 50 mM sodium
bicarbonate buffer (pH 9.6) containing 100 ng/well BSA plus 1 µg/well individual peptides.
Plates were blocked at room temperature for 2 h with 200 µl/well 5% sucrose, 1.25% nonfat
dry milk, and 2.5% BSA. Serum samples were diluted 1:100 or 1:5000 in Dilsim π, 100
µl/well were added to plates, and incubated for 1 h at 37°C. Plates were washed four times
with PBS plus 0.5% tween 20. Plates were incubated at 37°C with 100µl/well goat anti-
human IgG HRP conjugate diluted in PBS plus 20% NGS and 0.1% Triton-X-100 for 1 h.
Plates were washed four times in PBS + 0.5% tween 20. Plates were incubated for 30 min
at room temperature with 100µl/well TMB substrate, and stopped with 100 µl/well 2 N
sulfuric acid. Absorbance was read at 450 nm. Absorbance values from pre-bleed samples
were subtracted as background from the absorbance values of reactive samples.

ELISA assays were conducted on serum samples from immunized and control
animals both before and after challenge with SHTV98.6. Antibodies to Tat, Rev and VI
were obtained from Advanced BioScience Laboratories, Inc., 5510 Nicholson Lane,
Kensington, Maryland 20895.

**Results**

The data presented in Fig. 13A shows plasma virus levels following challenge
exposure in vaccinated animals (Fig. 13A) and controls (Fig. 13B). Controls consisted of
two animals challenged simultaneously with the vaccinated animals and four additional
animals that were historical controls challenged in exactly the same way using the identical
virus stock.
Three out of four animals of the vaccinated group were able to control viral replication. These animals can be ranked in their ability to control viral replication as 316 first, 308 second, and 490 third (Fig. 13A). As expected, these three animals were protected from CD4+ T cell loss (Fig. 14A).

The levels of SIV RNA as measured by copies/μg RNA in various tissues and control animals were assessed at the time of sacrifice. The level of SIV RNA in plasma was measured per 100 ul of plasma for immunized animals and 500 ul plasma for control animals. The results are shown in Figure 24. The results show that the immunized animals 308 M and 316 M that controlled viral replication had about 3 logs decrease of virus levels in lymph nodes, jejunum, spleen, and plasma as compared to control animals (320 M; 915 L). In contrast, the immunized animal 218 that did not control viral replication as well showed some decrease of 1-2 logs in viral levels in most tissues as compared to control animals.

This platform (DNA/protein boost) was chosen to favor the elicitation of antibodies over T cell response. Indeed, negligible ELISPOT responses were observed before (data not shown) and at the time of challenge (week 22). However, these responses were boosted by the challenge virus in the vaccinated animals (Fig. 15), especially in response to the 89.6 pool of p28TEV polypeptides. In control animals, no ELISPOT responses were observed, compatible with the fast and irreversible CD4 depletion that occurs following infection with 89.6P likely impairing the CD8+ T cell function. Animal 218 mounted a broad response to the Tev peptides derived from HIV_{BAL}, HIV_{SF2}, and HIV_{896P}, likely because it remained viremic during the observation time (Fig. 15). Animal 308 responded well to peptides from HIV_{89-6P} and less so to HTV_{BAL} and HRV_{SF2} (Fig. 15). Animals 316 responded poorly to all peptides in the pool, possibly because viral replication was blunted early. Correlative analysis of T cell response and the level of virus following challenge exposure demonstrated no correlation with T cell response and control of virus replication.

As our intention was to induce antibodies to V1, we measured the relative level of antibodies induced by our regimens in the sera of macaques before and after challenge exposure. ELISA titers were measured against the Tat, Rev, and the pool of V1 peptides (SFI 62, BAL, 89.6P). All vaccinated animals mounted titers of antibodies to Tat in the range of 1.2x10^4 to 3.5x10^4, and, at the time of challenge, they had equivalent titers of antibodies (see Fig. 16). Surprisingly, no anamnestic response to Tat was observed. Low levels of antibody to Rev were observed (Fig. 17), particularly in macaques 490 and 218. As in the case of Tat, Rev antibodies were not boosted by viral infection. In contrast, antibody titers to V1 ranging between 6x10^3 and 3.5x10^4 were elicited by vaccination and boosted by viral challenge in three of the macaques that were protected against high virus load in the long term, CD4+ T cell loss, and death. Macaque 218 that behaved like a control animal
had no detectable antibodies following challenge exposure (Fig. 18). Similarly, control macaques had background levels of antibodies to V1 (Fig. 18).

**Example 6**

To further confirm these data and to assess which region of V1 was reactive to the sera of the immunized macaques, we performed peptide scan analysis on all animal sera. 15-mer peptides, overlapping by 11 aa, derived from the cDNA amino acid sequence of the p28\textsuperscript{am} protein of 89.6 (see Figure 33) were synthesized. The purity of the peptides was >70%, as determined by HPLC and mass spectrophotometry, other contaminants being incomplete peptides. The peptides diluted in 5 mM HEPES (pH 7) were added to 96-well plates (Dynatech) in volumes of 10 µl per well at a final concentration of 0.3 µmolper well.

These assays demonstrated that, while all animals recognized similar peptides within Tat and Rev, they differed in their ability to respond to the V1 region (Figs. 19-23). Indeed, the three animals that fared better and were able to control viremia recognized peptides spanning 21-24, which correspond to the V1 region (Figs. 19-23). As expected, these peptides within the V1 region were not recognized by the sera of control animals 915 and 320 (Fig. 22). It is worth noting that the animal that had the highest reactivity to peptides 21, 22, and 25 (l:5xl \textsuperscript{6}) was animal 316 that fared better and was able to control virus replication by week 6 from SHF/89.6\textsubscript{exposure} (Fig. 21).

As we had immunized with a mixture of cDNA and boosted only with the SHIV\textsubscript{gg.ep} protein, we wished to investigate whether the reactivity of the animal sera was type-specific. The sera from animals 316, 308, and 490 recognized peptides 21-24 of the 89.6P V1 region (Fig. 23A). In contrast, peptides 21-24 from the HTV\textsubscript{BAL} and HrV\textsubscript{3P162} were not recognized by any of the animal sera (Figs. 23B andC).

The animals that mounted a V1 response following challenge exposure, 316, 308, and 490, appeared to better control viral replication and maintained normal levels of CD4\textsuperscript{+} T cells, suggesting that this type-specific antibody response may have played a role. To investigate the mechanism of protection, we performed neutralization assays using the TZM cell line that expressed the CD4 and CCR5 molecule and 89.6P, SF162, and ADA viruses. Low levels of neutralizing antibody titers to 89.6P, SF162.LS, and NL-ADAr\textsubscript{s} were found at week 30 in most of the animals (Table 2), and did not appear to relate to the ability to control viral replication as they were elicited also in animals 218 and 915 that failed to control viral replication.
Table 2: Neutralizing and ADCVT activity of the macaque sera

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*Values are the serum dilution at which relative luminescence units (RLUs) were reduced 50% compared to virus control wells (no test sample).
ND = Not done.
Example 7

**Antibody Dependent Cellular Cytotoxicity Viral Inhibition (ADCVI) Assay**

Serum from the immunized animals and control animals post challenge were analyzed for the ability to participate in antibody dependent cellular cytotoxicity of HIV infected cells.

**ADCVI assay**

The ADCVI assay was based on methods described previously for measles virus and HIV. PHA-stimulated human CD4+ lymphocyte target cells were infected with SHIV<sub>89</sub>6P at an MOI of approximately 0.02. After adsorption for 1 h, cells were washed, incubated in 5% CO<sub>2</sub> at 37°C for 72 h in medium and washed an additional two times. Infected target cells (5x10<sup>4</sup>) were next plated in 96-well round-bottom microliter plates, to which heat-inactivated test plasma and fresh human PBMC effector cells (effector:target [E:T] ratio of 10:1) were added. Plasma in the absence of effector cells was also tested for anti-viral activity. After 8 days incubation at 37°C in 5% CO<sub>2</sub>, supernatant fluid was collected and assayed for SIV p27 by ELISA (Zeptometrix, Buffalo, New York). Virus inhibition due to ADCVI was calculated as follows: % inhibition = 100(1 - ([p27p]/[p27n])), where [p27p] and [p27n] are the concentrations of p27 in supernatant fluid from wells containing SW-positive or -negative plasma, respectively.

The results are shown in Figure 25A. The results in Figure 25 show that plasma from at least two of the immunized animals 308 and 490 showed an increase antibody mediated cellular viral inhibition post challenge with SHIV 89.6P as measured by % decrease in expression of SIV p27. These two animals were effective at controlling viral replication. The immunized animal 316 showed a slight increase in ADCVI post challenge. One of the control animals also demonstrated an ADCVI response post challenge. These results indicate that antibodies specific to the VI region of p28<sub>TEV</sub> may in part control viral replication by an antibody dependent cellular viral inhibition mechanism.

When plasma samples from animals 308, 316, 490, and 915 (control) from week 26 were diluted and then tested for viral inhibition in the ADCVI assay, plasma from animals 308 and 316 at a dilution of 1:6400 showed 2-4 fold greater inhibition of viral replication than control animal 915 and immunized animal 490. (See Figure 25B) Plasma from animal 308 at 28 weeks at a dilution of 1:6400 shows viral inhibition at 80%. (Figure 25C) These results show that immunization of animals with p28 Tev induces antibodies that are capable of inhibiting virus via antibody dependent cytotoxicity.

It should be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.
All publications and patent applications in this specification are indicative of the level of ordinary skill in the art to which this disclosure pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated by reference.

The disclosure has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the disclosure.
WHAT I S CLAIMED IS:

1. A immunogenic composition comprising an effective amount of at least one isolated nucleic acid encoding at least one p28\textsuperscript{TCV} polypeptide having at least about 70 percent amino acid sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NO:151, SEQ ID NO:143, SEQ ID NO:146, SEQ ID NO:1, and fragments, including amino acids 75-110 of SEQ ID NO:151.

2. The immunogenic composition of claim 1, wherein the nucleic acid further encodes a second HIV antigen.

3. The immunogenic composition of claim 2, wherein the second HIV antigen is selected from the group consisting of Vif, Tat, Gag, Env, Rev, gp120, gp41, p24, p7, p17, or an immunogenic fragment thereof.

4. The immunogenic composition of claim 1, wherein the nucleic acid further encodes an immunomodulator for increasing the immunogenic response.

5. The immunogenic composition of claim 1, wherein the composition comprises at least one adjuvant.

6. The immunogenic composition of claim 1, wherein the nucleic acid comprises a recombinant viral vector.

7. The immunogenic composition of claim 1, wherein the nucleic acid encodes at least two different p28\textsuperscript{TEV} polypeptides from different HIV clades.

8. The immunogenic composition of claim 1, wherein the nucleic acid comprises a recombinant bacterial vector.

9. The immunogenic composition of claim 1, wherein the composition comprises at least two nucleic acids, each nucleic acid encoding a p28\textsuperscript{TCV} polypeptide from a different HIV clade.

10. The immunogenic composition of claim 1, wherein the nucleic acid is at least one naked DNA.
11. The immunogenic composition of claim 1, wherein the polypeptide comprises a sequence having at least about 80 percent amino acid sequence identity with an amino acid sequence of SEQ ID NO: 151.

12. The immunogenic composition of claim 1, wherein the polypeptide comprises an amino acid sequence having at least about 90 percent amino acid sequence identity with an amino acid sequence of SEQ ID NO: 151.

13. An immunogenic composition comprising an immunogenic effective amount of at least one isolated and purified polypeptide comprising an amino acid sequence having at least about 70 percent amino acid sequence identity with the p28\textsuperscript{TEV} polypeptide with the amino acid sequence selected from the group consisting of SEQ ID NO: 143, SEQ ID NO: 146, SEQ ID NO: 151, SEQ ID NO: 1, and fragments thereof.

14. The immunogenic composition of claim 13, wherein the polypeptide comprises an amino acid sequence having at least about 80 percent amino acid sequence identity with the polypeptide with the amino acid sequence of SEQ ID NO: 151.

15. The immunogenic composition of claim 13, wherein the polypeptide comprises an amino acid sequence having at least about 90 percent amino acid sequence identity with the polypeptide with the amino acid sequence of SEQ ID NO: 151.

16. The immunogenic composition of claim 13, wherein the polypeptide comprises an amino acid sequence of SEQ ID NO: 151.

17. The immunogenic composition of claim 13, comprising at least two isolated and purified p28\textsuperscript{TEV} polypeptides from at least two HIV-I clades.

18. The immunogenic composition of claim 13, further comprising an immunomodulator or adjuvant.

19. The immunogenic composition of claim 1, wherein the nucleic acid encodes a polypeptide that has at least 90\% sequence identity to the polypeptide comprising the sequence selected from the group consisting of SEQ ED NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, and mixtures thereof.
20. The immunogenic composition of claim 13, wherein the polypeptide has at least
90% sequence identity to the polypeptide comprising the sequence selected from the group
consisting of SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ
ID NO: 140, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID
NO: 156, and mixtures thereof.

21. A method for reducing HIV or SIV viral levels in a mammal comprising:
administering a composition of claim 1 to a mammal.

22. The method of claim 21, further comprising subsequently administering the
composition of claim 13 to the mammal.

23. An antibody that specifically binds: a) a naturally occurring p28\textsuperscript{TEV} polypeptide
comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 143,
SEQ ID NO: 146, SEQ ID NO: 151, SEQ ID NO: 152, and fragment thereof; or b) a polypeptide
having an amino acid sequence about 90% identical to a polypeptide comprising an amino
acid sequence of SEQ ID NO: 1, SEQ ID NO: 143, SEQ ID NO: 146, SEQ ID NO: 151, and
fragment thereof.

24. The antibody of claim 23, wherein the antibody is generated or selected using a
polypeptide having at least 90% sequence identity to the polypeptide comprising the
sequence selected from the group consisting of SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID
NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID
NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, and mixtures thereof.

25. An antagonist of p28\textsuperscript{TEV} polypeptide, comprising an isolated polynucleotide that
inhibits expression of p28\textsuperscript{TEV}.

26. The antagonist of claim 25, wherein the polynucleotide is an antisense RNA, small
interfering RNA (siRNA) molecule, or a microRNA molecule.

27. The antagonist of claim 25, wherein the polynucleotide hybridizes under moderately
stringent conditions to a nucleic acid molecule encoding p28\textsuperscript{TEV}.

28. The antagonist of claim 25, wherein the polynucleotide comprises a polynucleotide
sequence of SEQ ID NO: 2.
29. The antagonist of claim 25, wherein the polynucleotide hybridizes under moderately stringent conditions to a nucleic acid molecule encoding a p28\textsuperscript{TEV} splice acceptor site or p28\textsuperscript{TEV} splice donor site.

30. The antagonist of claim 29, wherein the nucleic acid sequence comprises a polynucleotide sequence encoding an Env exon.

31. The antagonist of claim 30, wherein the exon is exon 6D.

32. The antagonist of claim 29, wherein the p28\textsuperscript{TEV} splice acceptor site comprises a polynucleotide sequence of SEQ ID NO:3.

33. The antagonist of claim 29, wherein the p28\textsuperscript{TEV} splice donor site comprises a polynucleotide sequence of SEQ ID NO:4.

34. A vector comprising the polynucleotide of claim 25.

35. A host cell comprising the vector of claim 34.

36. A method for detecting HIV in a biological sample, comprising:
   (a) contacting a biological sample with at least one antibody that specifically binds a p28\textsuperscript{TEV} polypeptide of claim 23; and
   (b) detecting the presence of p28\textsuperscript{TEV} in the biological sample.

37. A method for identifying an antagonist of p28\textsuperscript{TEV} polypeptide, comprising:
   a) contacting a p28\textsuperscript{TEV} polypeptide or cell comprising a polynucleotide encoding a p28\textsuperscript{TEV} polypeptide cell with a candidate agent; and
   b) determining whether the candidate agent reduces HIV viral levels or transcriptional activity of the p28\textsuperscript{TEV} polypeptide, wherein the candidate agent that inhibits the HTV viral levels or transcriptional activity of p28\textsuperscript{TCV} polypeptide is identified as an antagonist of the p28\textsuperscript{TEV} polypeptide.

38. A method for inhibiting HIV viral levels, comprising administering to a patient in need thereof an effective amount of an antagonist of expression or activity of a p28\textsuperscript{TEV} polypeptide.

39. The method of claim 38, wherein the antagonist is an antibody.
40. The method of claim 39, wherein the antibody is humanized.

41. The method of claim 40, wherein the antibody binds to the V1 region of p28TEV.

42. The method of claim 41, wherein the antibody is attached to a siRNA molecule or cytotoxic molecule.

43. The method of claim 41, wherein the antibody is a Fv, Fab, Fab', or F(\text{ab}')_2 fragment.

44. The method of claim 38, wherein the antagonist is a polynucleotide that inhibits expression of p28TEV.

45. The method of claim 44, wherein the polynucleotide is an antisense RNA or a small interfering RNA (siRNA) molecule.

46. The method of claim 45, wherein the polynucleotide hybridizes under stringent conditions to a nucleic acid molecule encoding p28TEV.

47. The method of claim 46, wherein the nucleic acid molecule comprises a polynucleotide sequence of SEQ ID NO:2.

48. The method of claim 46, wherein the polynucleotide hybridizes under stringent conditions to a nucleic acid molecule encoding a p28TEV splice acceptor site or p28TEV splice donor site.

49. The method of claim 38, wherein the patient is infected with HIV.

50. The method of claim 21, further comprising administering an antiviral agent.

51. The method of claim 50, wherein the antiviral agent is selected from the group consisting of nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, or attachment inhibitors.

52. The antibody of claim 23, wherein the antibody is monoclonal.
53. The antibody of claim 52, wherein the antibody is a humanized antibody.

54. The antibody of claim 23, wherein the antibody is Fv Fab, Fab', or F(ab')₂.

55. The antibody of claim 23, wherein the antibody is attached to a detectable label, cytotoxic agent, or siRNA molecule.
FIG. 2C

p24 = pg/unit of TK Luc

pHxB2: - - + + + + +
p28²EV (µg): - - 0.5 1.0 5.0 10
RLTK: + + + + + + +
HIV-luc: + + + + + + +
HXB2 p28 TEV aa sequence

- MEPVDPRLEPWKHPGSQPKTAC
- TNCYCKKCCFHCQVCFITKALGI
- SYGRKRRQRRAHQNQSTHQ
- ASLSKHLKCTDLDNNTNTNSSS
- GRMIMEKGEIKNCFSNISTSIRD
- PPPNPQGRQARRRRRWRER
- QRQIHSISERILGTYLGRSAEPV
- PLQLPLLRLTLDCNEDCGTSGT
- QGVGPSQILVESPPTVLESGTKE
FIG. 5

1 atggagccag tagatctag actagagccc tggaagcatc caggaagtca
51 gctaaaaact gctttgtacca attgtatattg taaaaagttg tgcctttcatt
101 gcccaagtttg ttctcataca aagcctttag gcatctctta tggcaggaag
151 aagoggagac agcgacogaag agctcatcag aacagtccga ctcactcaagc
201 ttctotatca aacattttaa agtgcactga tttgaagaat gatactaata
251 ccaatagtga tagggggaga atgataatgg agaaaggaga gataaaaaac
301 tgcttcttca atatcagcac aagcataaga gacccacctc ccaatcaccga
351 ggggaccocga caggcccgga ggatagaaag aagaaggtgg agagagagac
401 agagacagat ccaattccgatt agtgaacggga tctttgcac ttatctggga
451 cgatctgcgg agcctgtgcc tcttcagcta ccaccgcttg agagacttac
501 tctttgatgt aacgaggatt gtgggaacttc tgggacgcag ggggtgggaa
551 gccctcaaat atggttggaa tctcctacag tattggaagtca aggaactaaa
601 caatag
FIG. 10

48h

5 4 3 2 1 0
p24 pg/ml

24h

2.5 2 1.5 1 0.5 0
p24 pg/ml

pXB2
pXB2Δtev

α-tubulin

gp160 gp120 p55 p42 p24

Rev
FIG. 16
FIG. 21

89.6 Tev Pepscans: Rhesus Macaque 316 Serum (1:5000)

316

wk10

wk19

wk24

wk27
FIG. 34A
Plasmid Map:

040055pPCR-Script
3508 bp

FIG. 34B
Plasmid Map:

040054pPCR-Script
3490 bp