HIV phylogenic classifications are normally based either on nucleotide sequences derived from multiple sub genomic regions (*gag, pol* and *env*) of the same isolates, or on full-length genome sequence analysis. A phylogenic analysis of HIV-1 near-full length sequences revealed that HIV-1 subtype B was most closely related genetically to HIV subtype D (Figure 1). Phylogenic analyses of HIV-1 Gag and Pol protein sequences also showed that HIV-1 subtype B was most closely related genetically to HIV subtype D (Figure 2).

Nevertheless, HIV-1-NDK, a subtype D virus, is significantly more cytopathic for CD4+ lymphocytes than the HIV-1-BRU prototype, a subtype B virus. This may be due to enhanced fusogenicity and infectivity of subtype D viruses. De Mareuil et al., J. Virol. 66: 6797 (1992). Phenotypic analysis of recombinant viruses indicated that 75 amino acids from the N-terminal part of HIV-1-NDK matrix (MA) protein, together with the HIV-1-NDK envelope glycoprotein, are responsible for enhanced fusogenicity of HIV-1-NDK in CD4+ lymphocytes as well as for enhanced infectivity of HIV-1-NDK in some CD4- cell lines. *Id.*

There is a need in the art for lentiviral packaging constructs producing higher titers of packaged lentiviral vectors, in order to reduce injection volumes, increase dosages, reduce the cost of vaccination, and increase the number of patients that could be treated with one batch. The current invention fulfills this need.

Brief Summary ~~of the Invention~~

The *gag-pol* gene of subtype B of HIV-1 in a lentiviral packaging plasmid (construct p8.74) was replaced by the *gag-pol* gene of a subtype D HIV-1 to generate construct pThV-GP-N. The constructs were used for lentiviral vector production. Approximately 2-fold higher titers were obtained using the pThV-GP-N plasmid as compared to construct p8.74. Thus, the Gag-Pol of a subtype D virus increases the titer of lentiviral vector particles relative to a Gag-Pol of a subtype B virus.

In one aspect, there is provided a replication-defective lentiviral packaging vector lacking a Ψ site and encoding a subtype D HIV-1 Gag-Pol protein.

In another aspect, there is provided a method for generating a packaging vector, the

method comprising inserting a nucleotide sequence encoding a subtype D HIV-1 Gag-Pol protein into a plasmid under the control of a non-HIV promoter to generate the packaging vector.

In another aspect, there is provided an *in vitro* method for generating a lentiviral vector particle, the method comprising administering a packaging vector encoding a subtype D HIV-1 Gag-Pol protein to a cell with a lentiviral vector.

In another aspect, there is provided a cell comprising the vector as described herein.

In another aspect, there is provided a lentiviral vector particle comprising subtype D HIV-1 Gag and Pol proteins.

~~The invention e~~Encompassed herein is a lentiviral packaging vector comprising a subtype D *gag-pol* sequence, particularly from HIV-1 NDK. In ~~a preferred embodiment~~one example, the lentiviral packaging vector comprises the nucleotide sequence of SEQ ID NO:1. In ~~a preferred embodiment~~another example, the lentiviral packaging vector encodes the amino acid sequence of SEQ ID NO:2.

The nucleotide sequence of SEQ ID NO 1 is:

atgggtgcgagagcgtcagtattaagcgggggaaaattagatacatgggaaagaattcggttac
ggccaggaggaaagaaaaaatatgcactaaaacatttgatatgggcaagcagggagctagaacg

MGARASVLSGGKLDAWERIRLRPGGKKKYALKHLIWASRELERIALNPGLLETSEGCK
QIIGQLQPSIQTGSEELRSLYNTIATLYCVHERIEVKDTKEAVEKMEEEQNKSKKKTQQA
AADSSQVSQNYPIVQNLQGQMVHQAISPRTLNAWVKVIEEKAFSPEVIPMFSALSEGA
TPQDLNTMLNTVGGHQAAMQMLKETINDEAAEWDRLHPVHAGPVAPGQMREPRGSD
IAGTTSTLQEQIAWMTSNPPIPVGEIYKRWIILGLNKIVRMYSPVSILDIRQGPKEPFRDY
VDRFYKTLRAEQASQDVKNWMTETLLVQNANPDCKTILKALGPQATLEEMMTACQGV
GGPGHKARVLAEAMSQVTGSVTAVMMQRGNFKGPRKSIKCFNCGKEGHTAKNCRAP
RKKGCWKCGREGHQMKDCSERQANFLGKIWPSHKGRPGNFLQSRPEPTAPPAESF
GFGEEITPSQKQEQKDKELYPLASLKSLFGNDPSSQFFREDLAFPQGKAGEFSSEQTR
ANSPTSRELRVWGGDNPLSETGAEGQGTVSFSFPQITLWQRPLVTIKIGGQLKEALLD
TGADDTVLEEMNLPGKWKPKMIGGIGGFIKVRQYDQILIEICGYKAMGTVLVGPTPVNII
GRNLLTQIGCTLNFPISPIETVPVKLKPGMDGPKVKQWPLTEEKIKALTEICTEMEKEGK
ISRIGPENPYNTPIFAIKKKDSTKWRKLVDFRELNKRTQDFWEVQLGIPHPAGLKKKKS
VTVLDVGDAYFSVPLDEDFRKYTAFTIPSINNETPGIRYQYNVLPQGWKGSPAIFQSSM
TKILEPFRKQNPEIVIYQYMDDLYVGSDLEIGQHRTKIEELREHLLRWGFTTPDKKHQKE
PPFLWMGYELHPDKWTVQPIKLPEKESWTVNDIQKLVGKLNWASQIYAGIKVKQLCKL
LRGTKALTEVVPLTEEAELELAENREILKEPVHGVYYDPSKDLIAELQKQGDGQWTYQI
YQEPFKNLKTGKYARTRGAHTNDVKQLTEAVQKIATESIVIWGKTPKFKLPIQKETWET
WWIEYWQATWIPEWEFVNTPPLVKLWYQLEKEPIIGAETFYVDGAANRETKLGKAGYV
TDRGRQKVVPFTDTTNQKTELQAINLALQDSGLEVNIVTDSQYALGIIQAQPDKSESEL
VSQIIEQLIKKEKVYLAWVPAHKGIGGNEQVDKLVSQGIRKVLFLDGIDKAQEEHEKYHN
NWRAMASDFNLPPVVAKEIVASCDKCQLKGEAMHGQVDCSPGIWQLDCTHLEGKVIL
VAVHVASGYIEAEVIPAETGQETAYFLLKLAGRWPVKVVHTDNGSNFTSATVKAACWW
AGIKQEFGIPYNPQSQGVVESMNKELKKIIGQVRDQAEHLKTAVQMAVFIHNFKRKGGI
GGYSAGERIIDIIATDIQTRELQKQIIKIQNFRVYYRDSRDPIWKGPAKLLWKGEGAVVIQ
DNSDIKVVPRRKVKIIRDYGKQMAGDDCVASRQDED (SEQ ID NO:2).

~~The invention~~ Eencompasseds herein is a lentiviral packaging vector comprising a subtype D MA sequence, particularly from HIV-1 NDK. In ~~a preferred embodiment~~one example, the lentiviral packaging vector encodes an MA protein comprising the amino acid sequence of SEQ ID NO:3.

The amino acid sequence of SEQ ID NO 3 is:

MGARASVLSGGKLDTWERIRLRPGGKKKYALKHLIWASRELERFTLNPGLLETSEGCK
QIIGQLQPSIQTGSEEIRSLYNTVATLYCVHERIEVKDTKEAVEKMEEEQNKSKKKTQQ
AAADSSQVSQNY (SEQ ID NO 3).

Preferably, the lentiviral packaging vector encoding the HIV Gag MA protein generates at least a 1.5 fold increase, or at least a 2-fold increase, in the titer of a packaged lentiviral vector as compared to the lentiviral packaging vector encoding an HIV Gag MA protein of HIV-1 BRU, e.g., p8.74. Preferably, the lentiviral packaging vector is replication-defective and lacks a Ψ site.

In ~~a preferred embodiment~~one example, the lentiviral packaging vector encodes an HIV Gag MA protein having an amino acid at position 12 that is not a glutamic acid and an amino acid at position 15 that is not an arginine. Preferably, the lentiviral packaging vector does not have both a valine at position 46 and a leucine at position 61.

In ~~a preferred embodiment~~another example, the amino acid at position 12 of the MA protein is a lysine. In a ~~preferred embodiment~~further example, the amino acid at position 15 is a threonine. In a ~~preferred embodiment~~further example, the amino acid at position 15 is an alanine. In ~~a preferred embodiment~~one example, the amino acid at position 46 is a leucine. In ~~a preferred embodiment~~one example, the amino acid at position 61 is an isoleucine. In ~~a preferred embodiment~~one example, the amino acid at position 61 is a methionine.

In one ~~embodiment~~example, the vector does not encode a functional Env protein.

~~The invention A~~also ~~e~~encompasse~~d~~s herein are methods for making the above lentiviral packaging vectors and methods for using these lentiviral packaging vectors.

Brief Description of the Drawings

The invention is more fully understood through reference to the drawings.

Fig. 1 depicts phylogenic trees of HIV viruses.

Fig. 2A and B depict phylogenic trees of HIV GAG (A) and POL (B) proteins.

GAG and POL protein sequences were obtained from: http://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html. For each known HIV clade, one patient's (for clade B) or two patients' virus sequences were randomly chosen and GAG and POL protein sequences were compared to the reference clade B proteins (B.FR.83.HXB2_LAI_IIIB_BRU.KO3455). Alignments were performed using Vector NTI advance 11 (Invitrogen).

Fig. 3 depicts titers obtained using the p8.74 and the pThV-GP-N plasmids for vector production. Lentiviral particles were produced using the proviral plasmid (pFLAP-CMV-GFP), the pseudotyping plasmid (pTHV-VSV.G) and either the commonly used packaging plasmid (derived from the BRU strain, p8,74) or an NDK-derived packaging plasmid (pTHV-GP-N). With each packaging plasmid, 18 independent transfections were performed and the particles titers were measured by FACS analysis. Similar

Claims

We claim:

1. A replication-defective lentiviral packaging vector lacking a Ψ site and encoding an HIV-1 Gag MA protein;

wherein the HIV-1 Gag MA protein:

a) has an amino acid at position 12 that is not a glutamic acid,

b) has an amino acid at position 15 that is not an arginine,

c) does not have both a valine at position 46 and a leucine at position 61; and

wherein the lentiviral packaging vector encoding the HIV-1 Gag MA protein generates at least a 1.5 fold increase in the titer of a packaged lentiviral vector as compared to the lentiviral packaging vector encoding an HIV-1 Gag MA protein of HIV-1 BRU.

2. The vector of claim 1, wherein the amino acid at position 12 is a lysine.

3. The vector of claim 1, wherein the amino acid at position 12 is an arginine.

4. The vector of claim 1, wherein the amino acid at position 15 is a threonine.

5. The vector of claim 1, wherein the amino acid at position 15 is an alanine.

6. The vector of claim 1, wherein the amino acid at position 46 is a leucine.

7. The vector of claim 1, wherein the amino acid at position 61 is an isoleucine.

8. The vector of claim 1, wherein the amino acid at position 61 is a methionine.

9. The vector of claim 2, wherein the amino acid at position 15 is a threonine.

11. The vector of claim 2, wherein the amino acid at position 46 is a leucine.

12. The vector of claim 2, wherein the amino acid at position 61 is an isoleucine.

13. The vector of claim 9, wherein the amino acid at position 46 is a leucine.

14. The vector of claim 9, wherein the amino acid at position 61 is an isoleucine.

15. The vector of claim 13, wherein the amino acid at position 61 is an isoleucine.

16. The vector of claim 15, wherein the HIV-1 Gag MA protein is the HIV-1 Gag MA protein of HIV-1 NDK.

17. The vector of claim 16, wherein the vector encodes the amino acid sequence of SEQ ID NO:4.

18. A method for generating a packaging vector comprising inserting a Gag and/or Pol sequence from a non-subtype B HIV-1 virus into a plasmid under the control of a non-HIV promoter to generate the packaging vector.

19. A method for generating a lentiviral vector comprising administering a packaging vector encoding a non-subtype B HIV-1 Gag or Pol protein to a cell with a lentiviral vector.

20. The method of claim 19, further comprising selecting for a packaging vector that packages a higher titer of the lentiviral vector than a same packaging vector encoding a subtype B HIV-1 Gag or Pol protein.

Claims

1. A replication-defective lentiviral packaging vector lacking a Ψ site and encoding a subtype D HIV-1 Gag-Pol protein.

2. The vector of claim 1, wherein the HIV-1 Gag-Pol protein is the HIV-1 Gag-Pol protein of HIV-1 NDK.

3. The vector of claim 2, wherein the vector encodes the amino acid sequence of SEQ ID NO: 2.

4. The vector of any one of claims 1 to 3, wherein the vector is a plasmid comprising a nucleotide sequence encoding a subtype D HIV-1 Gag-Pol protein.

5. A method for generating a packaging vector, the method comprising inserting a nucleotide sequence encoding a subtype D HIV-1 Gag-Pol protein into a plasmid under the control of a non-HIV promoter to generate the packaging vector.

6. The method of claim 5, wherein the HIV-1 Gag-Pol protein is the HIV-1 Gag-Pol protein of HIV-1 NDK.

7. The method of claim 6, wherein the vector encodes the amino acid sequence of SEQ ID NO: 2.

8. An *in vitro* method for generating a lentiviral vector particle, the method comprising administering a packaging vector encoding a subtype D HIV-1 Gag-Pol protein to a cell with a lentiviral vector.

9. The method of claim 8, wherein the packaging vector and the lentiviral vector are plasmids.

10. The method of claim 8, wherein the cell transiently expresses subtype D HIV-1 Gag and Pol proteins.

11. The method of claim 8, wherein the packaging vector is integrated into the genome of the cell.

12. The method of claim 8, wherein the HIV-1 Gag-Pol protein is the HIV-1 Gag-Pol protein of HIV-1 NDK.

13. The method of claim 12, wherein the packaging vector encodes the amino acid sequence of SEQ ID NO: 2.

14. The method of claim 8, further comprising selecting for a packaging vector that packages a higher titer of the lentiviral vector than a same packaging vector encoding a subtype B HIV-1 Gag-Pol protein.

15. A cell comprising the vector of any one of claims 1-3.

16. The cell of claim 15, wherein the vector is integrated into the genome of the cell.

17. A lentiviral vector particle comprising subtype D HIV-1 Gag and Pol proteins.

18. The lentiviral vector particle of claim 17, wherein the Gag and Pol proteins are HIV-1 NDK Gag and Pol proteins.

19. The method of claim 18, wherein the Gag and Pol proteins are Gag and Pol proteins produced by expressing a nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 2.