Institute of Technology and cloned into the pcDNA3 vector (Invitrogen) by PCR to generate plasmid pSVG. A ten-residue tag sequence was inserted into E2 protein between amino acids 71 and 74 by PCR mutagenesis to disrupt the HS binding site. An additional deletion was introduced to the E3 glycoprotein of SVG to remove amino acids 61-64. This modified SVG was designated as SVGmu (SEQ ID NO:11 of WO2008/011636). The cDNA for SVGmu was cloned downstream of the CMV promoter in the pcDNA3 vector (designated as pSVGmu, SEQ ID NO: 3 of WO2008/011636).

SUMMARY~~SOME EMBODIMENTS OF THE PRESENT INVENTION~~

[0008] Though the SVGmu pseudotyped viral particles were able to selectively transduce cells expressing the DC-SIGN antigen, several aspects of the system make it unsuitable for therapeutic use. For example, the E3/E2 fusion protein displays an antigenic epitope of influenza hemagglutinin, the virus genome integrates into the host chromosome, which can activate deleterious host genes, and the present inventors have found that titers of the virus were low compared to the titer of particles pseudotyped with a VSV-G envelope. Significantly, Sindbis virus strains with a mutation preventing correct processing of E3 from the E2 glycoprotein (so-called "pE2 mutants"), such as SVGmu, grow poorly in permissive cell lines and are severely attenuated in mouse pathogenicity.

[0009] An alignment of the SVGmu E3-E2 protein used in WO2008/011636 to pseudotype a lentiviral vector against three exemplary E protein variants of the present ~~invention~~disclosure is shown in Figure 1. The numbering indicated in Figure 1 is by reference to the HR strain of Sindbis virus envelope proteins. Unless otherwise indicated, numbering used herein is by reference to this strain of Sindbis virus. The present inventors have modified the SVGmu protein to provide an improved lentiviral production system. The viral particles of the ~~invention~~disclosure are produced in a significantly higher titre than those using SVGmu, and also stimulate a stronger immune response. In addition, the

pseudotyped lentiviral particles have improved titre and improved infectivity whilst retaining selectivity for DCs.

[0009a] In one aspect, there is provided a lentiviral vector particle comprising:

(a) an envelope comprising

(i) a Sindbis virus E2 glycoprotein of SEQ ID NO:1 in which 160X is absent or is an amino acid other than glutamic acid, or a variant of SEQ ID NO:1 thereof having at least 80% identity to SEQ ID NO:1 and in which 160X is absent or is an amino acid other than glutamic acid, capable of infecting dendritic cells;

wherein E2 is not part of a fusion protein with Sindbis virus E3; and

(b) a lentiviral vector genome comprising one or more sequences of interest.

[0010] In one embodiment, the ~~invention~~disclosure provides a retroviral, such as a lentiviral, vector particle comprising: (a) an envelope comprising a Sindbis virus E2 glycoprotein of SEQ ID NO:1 in which 160X is absent or a non-acidic amino acid, or a variant of SEQ ID NO:1 capable of infecting dendritic cells; wherein said E2 glycoprotein or variant thereof is not fused to E3; and (b) a lentiviral genome comprising a sequence of interest.

[0011] The variant may be capable of binding to DC-SIGN. Preferably it also has reduced binding to heparan sulfate compared to a reference protein from HR strain. The E2 protein of the HR strain is shown as SEQ ID NO: 18.

[0012] Preferably, 160X is one of the small or aliphatic amino acids, including glycine, alanine, valine, leucine or isoleucine. In one ~~aspect~~embodiment, 160X is absent or is glycine, valine, leucine or isoleucine. In one embodiment, X is glycine.

[0013] A variant of SEQ ID NO:1 is defined as comprising a sequence having at least 80% sequence identity, or at least 82%, 85%, 87%, 90%, 92%, 95%, or 98% sequence identity to SEQ ID NO:1 in which 160X is retained and as defined above. The variant may have one or more of the lysines and arginines in the region spanning residues 50 to 180 independently deleted or substituted with a non-basic amino acid. In one embodiment, the non-basic amino acid is glutamic acid or aspartic acid.

[0014] In one ~~aspect~~embodiment, residue X is selected from a deletion, glycine, valine, leucine or isoleucine and one or more of the lysines and arginines in the region spanning residues 50 to 180 are independently deleted or substituted with a non-basic amino acid.

[0015] In another ~~aspect~~embodiment, X is selected from a deletion, glycine, alanine, valine, leucine or isoleucine and one or more of the lysines and arginines in the region spanning residues 50 to 180, preferably including at

specificity for paired arginine residues that are separated by two amino acids. To maintain E3/E2 cleavage by furin, residues 62-66 (RSKRS; SEQ ID NO: 26) should maintain the two arginine residues with two amino acid separation and the serine residue.

[0021] In one embodiment of the ~~invention~~disclosure, the polyprotein comprises an E3 sequence corresponding to residues 1-65 of SEQ ID NO:20, or a variant thereof having at least 80% sequence identity, or at least 82%, 85%, 87%, 90%, 92%, 95%, or 98% sequence identity to residues 1-65 of SEQ ID NO:20, wherein residues 62-65 are RSKR (SEQ ID NO: 27) and the variant is capable of being incorporated into a pseudotyped viral envelope. Preferably, the E2 part of the polyprotein is any of the embodiments as defined herein above.

[0022] Alternatively, a different cleavage sequence can be used in place of the E3/E2 furin cleavage sequence or any of the other cleavage sequences. Recognition and cleavage sites can be incorporated for endopeptidases, including, without limitation, aspartic endopeptidases (e.g., cathepsin D, chymosin, HIV protease), cysteine endopeptidases (bromelains, papain, calpain), metalloendopeptidases, (e.g., collagenase, thermolysin), serine endopeptidases (e.g., chymotrypsin, factor IXa, factor X, thrombin, trypsin), streptokinases. The recognition and cleavage site sequences for these enzymes are well known.

[0023] Where such a cleavage site is used, it may be introduced into an E3 polyprotein comprising at least residues 1-60, such as residues 1-61 or 1-62 of SEQ ID NO:20 or a variant thereof having at least 80% sequence identity, or at least 82%, 85%, 87%, 90%, 92%, 95%, or 98% sequence identity to the corresponding number of residues of SEQ ID NO:20, fused directly at the C-terminal to said cleavage sequence, which in turn is fused to the E2 part of the polyprotein. Preferably, the E2 part of the polyprotein is any of the embodiments as defined herein above.

[0024] E2 protein sequences of the ~~invention~~disclosure include variants of SEQ ID NO:1 in which the 160X is as defined in the following Table, and protein

is as set out in SEQ ID NO:1 apart from the following residues 70, 76 and 159 and 160, which are as follows:

SEQ ID No:	70	76	159	*160X*
3	E	**K**	E	*G*
4	E	E	E	*G*
5	E	**K**	E	*Δ*
6	E	E	E	*Δ*
7	**K**	**K**	E	*G*
8	**K**	**K**	E	*Δ*
9	**K**	E	E	*G*
10	**K**	E	E	*Δ*
11	**K**	E	**K**	*G*
12	**K**	E	**K**	*Δ*
13	E	**K**	**K**	*G*
14	E	**K**	**K**	*Δ*
15	E	E	**K**	*G*
16	*E*	*E*	***K***	*Δ*

[0025] Optionally, each of the above sequences may comprise one or more further changes to SEQ ID NO:1 but have residues 71-75 of SEQ ID NO:1 that are unchanged, or may have one or two substitutions which do not affect the ability of the variant to infect DCs, but do not alter the number of *amino acids in this region.*

[0026] Further E2 protein sequences of the ~~invention~~disclosure comprise SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 or SEQ ID NO: 15 in which residue 160 is Ala, Ile, Leu or Val in place of Gly. Such variants may also comprise residues 71-75 of SEQ ID NO:1 that are unchanged, or may have one or two substitutions which do not affect

the ability of the variant to infect DCs, but do not alter the number of amino acids in this region.

[0027] Optionally the E2 sequence of SEQ ID NOs: 3-15, or the further variants described in the above two paragraphs, may be varied at other positions to provide an E2 sequence having at least 80% sequence identity to SEQ ID NO:1. "At least 80% sequence identity" includes any one of at least 82%, 85%, 87%, 90%, 92%, 95%, or 98% sequence identity.

[0028] *In the above embodiments, the first E2 residue,* corresponding to position 66 of SEQ ID NO:20, may be Ser.

[0029] As indicated above, the E2 sequences of the ~~invention~~disclosure, including any of SEQ ID NOs: 3-15 and the variants thereof described in the preceding three paragraphs, may be expressed from a polyprotein comprising at least an E3/E2 sequence, and preferably a Sindbis E3/E2/6K/E1 polyprotein sequence. The E3 polyprotein sequence in each of the embodiments may be that of residues 1-65 of SEQ ID NO:20 or any of the variants thereof described above, including variants with a non-native cleavage site.

~~SUMMARY OF THE INVENTION~~

[0030] This patent application is directed to pseudotyped lentiviral vectors that comprise a genome having a sequence of interest and an envelope *comprising a glycoprotein of an arbovirus. The arbovirus glycoprotein can be* from Sindbis virus, Dengue virus, and Venezuelan equine encephalitis virus. In particular, when the glycoprotein is an E2 protein from Sindbis virus, the E2 protein has at least one amino acid alteration at residue 160 compared to a SEQ ID NO: 1. The amino acid alteration can be a deletion or an amino acid other than glutamic acid. The E2 glycoprotein can alternatively be a variant protein that has at least 80% sequence identity to SEQ ID NO: 1 and also have an alteration of residue 160 that is either a deletion or an amino acid other than glutamic acid. The glycoprotein facilitates infection of dendritic cells. In all cases the E2 glycoprotein is not part of a fusion protein with Sindbis virus E3. In some embodiments, the E2 glycoprotein or variant binds DC-SIGN. The

lentiviral vector also comprises a lentiviral genome that comprises a sequence of interest.

[0031] In certain embodiments, residue 160 is absent or is glycine, alanine, valine, leucine or isoleucine. In one embodiment, residue 160 is glycine. In addition, other alterations of E2 glycoprotein can occur in combination with the mentioned alterations of residue 160. One such alteration is a change of an amino acid to reduce the net positive charge of E2. One way to reduce the net positive charge is to change one of the lysines to an amino acid that is not basic. In particular embodiments, one or more of lysine 70, lysine 76 or lysine 159 is altered. In certain embodiments, one or more of these lysines is changed to a glutamic acid or an aspartic acid. In specific embodiments, the E2 glycoprotein is one of SEQ ID NOs: 3-16. Examples of combinations of alterations include, without limitation, an alteration of glutamic acid at position 160, and alteration of one or more of lysine 70, lysine 76, or lysine 159 to a non-basic residue. Other alterations may be also be made, in combination with the alteration of residue 160 and optionally any of the other alterations disclosed herein. For example, any of the previously mentioned E2 glycoproteins may optionally comprise one or more further substitutions, insertions or deletions. As one specific example, the protein cleavage site between E2 and E3 can be either the native sequence or an altered sequence that is cleaved by a different endopeptidase. In other embodiments, which can be in combination with any of the above, the sequence of residues 71-75 of SEQ ID NO:1 is unchanged or has one or two amino acid substitutions which do not affect the ability of the variant to infect DCs.

[0032] In certain embodiments, the lentiviral vector genome of any of the foregoing viral particles comprises a sequence of interest that encodes a tumor-specific antigen or a virus-derived antigen, such as an HIV or SIV antigen. In some embodiments, any of the vector particles described are produced at a titer of at least 10^5/mL IU.

[0032a] In one aspect, there is provided a composition comprising a lentiviral vector particle as defined above, wherein the composition has an IU of at least 10^5/mL.

[0032b] In one aspect, there is provided a lentiviral vector packaging system for producing a pseudotyped lentiviral vector particle as defined above, or the composition as defined above, comprising:

(i) a first nucleic acid molecule encoding the Sindbis virus E2 glycoprotein as defined above (a)(i), and;

(ii) a second nucleic acid molecule encoding gag and pol proteins,

(iii) a third nucleic acid molecule encoding rev;

(iv) a lentiviral vector genome comprising a sequence of interest; and

(v) a packaging cell.

[0033] In another ~~aspect~~embodiment, a lentiviral vector packaging system for producing a pseudotyped lentiviral vector particle is provided, comprising: a first

nucleic acid molecule encoding a Sindbis virus E2 glycoprotein of SEQ ID NO:1 in which residue 160 is absent or an amino acid other than glutamic acid, or a variant thereof capable of infecting dendritic cells having at least sequence 80% identity to SEQ ID NO:1 and in which residue 160 is absent or is an amino acid other than glutamic acid, a second nucleic acid molecule encoding gag and pol proteins; a third nucleic acid molecule encoding rev; and a lentiviral vector genome comprising a sequence of interest. The E2 glycoprotein or variant of the packaging system has an amino acid sequence as defined in any of the embodiments above. In some embodiments, the pol protein has a non-functional integrase. In a particular embodiment, the non-functional Integrase has a D64V mutation. In some embodiments, the second nucleic acid molecule is a non-integrating lentiviral genome. In particular embodiments, the *att* site is mutated or deleted or the PPT site is mutated or deleted or both. The non-integrating lentiviral genome can be used in combination with a non-functional Integrase and in combination with any of the E2 proteins or variants. It is preferred that lentiviral vector particles are produced to a titer of at least 10^5 IU/mL. In some instances, a cell is transfected with the first and fourth nucleic acid molecules described above. The cell may already comprise the second and third nucleic acid molecules, in a stable transformation.

[0033a] In one aspect, there is provided an isolated nucleic acid molecule comprising a nucleotide sequence encoding the E2 glycoprotein or variant as defined above (a)(i), or as defined above.

[0034] An isolated nucleic acid molecule is provided that encodes the glycoprotein as described above or an E3/E2 glycoprotein optionally in the form of a Sindbis E3/E2/6K/E1 polyprotein, or an E3/E2 glycoprotein wherein the E3 sequence corresponds to residues 1-65 of SEQ ID NO:20, or a variant thereof having at least 80% sequence identity to residues 1-65 of SEQ ID NO:20, wherein residues 62-65 are RSKR (SEQ ID NO: 27) and the variant is capable of being incorporated into a pseudotyped viral envelope, optionally further wherein residue 1 of the E2 polyprotein is Ser. The E2 glycoprotein can be any of the variants described above, including the combinations of alterations. In addition, an expression vector comprising the nucleic acid molecule is provided, as is a host cell comprising the expression vector.

[0034a] In one aspect, there is provided a method of making a lentiviral vector particle as defined above, or the composition as defined above, comprising culturing the packaging cell as defined above.

[0035] A method of making a lentiviral vector particle of any one of the variants or combinations above is provided, comprising expressing in a cell a first nucleic acid molecule encoding a Sindbis virus E2 glycoprotein of SEQ ID NO:1 in which residue 160 is other than glutamic acid, or a variant thereof capable of infecting dendritic cells having at least sequence 80% identity to SEQ ID NO:1 and in which residue 160 is absent or is an amino acid other than glutamic acid, and; (ii) a second nucleic acid molecule wherein the second nucleic acid molecule can be transcribed and the transcript assembled into a pseudotyped lentiviral vector particle.

[0035a] In one aspect, there is provided a therapeutic or prophylactic vaccine comprising the lentiviral vector particles as defined above, or the composition as defined above, and a pharmaceutically acceptable excipient.

[0036] Any of the lentiviral vector particles can be used in a method of treatment of a human or animal subject. The treatment can be a vaccine for immunization, in which the vaccine is prophylactic or therapeutic. A vaccine comprises the lentiviral vector particle with a pharmaceutically acceptable excipient. Alternatively, lentiviral vector particles can be administered to cells in vitro, comprising mixing the cells with any of the lentiviral vector particles above.

[0036a] In one aspect, there is provided a lentiviral vector particle as defined above, or the composition as defined above, for use in a method of treatment of a human or animal subject, optionally comprising delivering lentiviral vector particles to dendritic cells ex vivo.

[0036b] In one aspect, there is provided a lentiviral vector particle as defined above, or the composition as defined above, for use in inducing an antigen-specific immune response, optionally a humoral response and/or cellular response.

[0037] These and other ~~aspects~~embodiments of the present ~~invention~~disclosure will become evident upon reference to the following detailed description and attached drawings. In addition, U.S. application number 61/228,491 filed on July 24, 2009 is incorporated by reference herein in its entirety for all purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] Figure 1 is a sequence alignment of the envelope protein for four Sindbis virus envelopes, SVGmu, SIN-Var1, SIN-Var2, and SIN-Var3. The alignment is shown relative to SVGmu, a previously described Sindbis envelope. Main differences to SVGmu include the regeneration of the furin like protease cleavage site (RSKR; SEQ ID NO: 27) between E3 and E2, removal of the HA epitope tag, and a series of lysine substitutions to reduce heparin binding.

[0039] Figure 2 is a schematic of exemplary vectors used in packaging viral particles.