A LENTIVIRAL VECTOR-BASED VACCINE

DESCRIPTION OF THE INVENTION

Field of the Invention

The invention is directed to lentiviral vector, methods of gene transfer, immunization, and vaccination using lentiviral vector, processes for the production of recombinant polypeptides, antibodies generated against these polypeptides, and the use of such molecules in diagnostic methods, kits, immunogenic compositions, vaccines, and antiviral therapy.

Background of the Invention

In the past few years, lentiviral gene transfer vectors have gained a considerable interest among the gene therapy community. This unmatched reputation is due to their ability to efficiently and stably transfer therapeutic or reporter genes to a large variety of cells and tissues of key importance for therapeutic intervention, such as hematopoietic stem cells, brain, liver and retina (*see* [1-4], among others). Lentiviral vectors achieve high transduction efficiency irrespective of the proliferative status of the target cells, thus circumventing one of the main limitations of oncovirus-derived retroviral vectors in which transduction is restricted to dividing cells.

This advantage is reflected in a number of successful preclinical tests in various animal models of human diseases (*see* [5-11], among others), and will undoubtedly translate into their exploitation in a growing number of clinical trials.

More recently, lentiviral vectors have also been proven as promising vaccination vectors. Most studies to date have focused on the induction of cellular immune responses in the field of anti-tumoral immunotherapy

[12-18], a few have also focused on the induction of protective cellular immunity against viruses [19-21].

Their capacity to transduce non-dividing dendritic cells (DCs) with high efficiency, *ex-vivo* [12, 14, 15, 19] as well as *in vivo* [13, 18, 22], accounts for their ability to elicit strong CTL responses.

Indeed, reports using lentiviral vectors to stimulate anti-tumor immunity show very promising results. Human DCs transduced by lentiviral vectors expressing tumor antigens stimulate specific CTL responses *in vitro* [12, 14, 15, 18]. In mice, the injection of lentiviral vector particles or lentiviral vector transduced DCs induce strong and specific anti-tumor cellular immune responses [13, 15, 18] and confer protection from tumor challenge [12, 17]. These responses are more potent than the ones obtained by classical immunization with peptide plus adjuvant [13] or even peptide-pulsed antigen presenting cells (APCs) whether assayed *ex vivo* [20] or *in vivo* [16, 21]. The observed advantage could be due to the continuous presentation of the antigen in vector-transduced APCs in contrast to the transient antigen presentation that ensues from peptide pulsing of APCs [16, 20].

To date, little is known of the ability of lentiviral vectors to stimulate an antibody-based protective immunity. Nevertheless, their high transduction efficiency could also grant them a strong ability to induce humoral immunity. Moreover, their seemingly preferential tropism for DCs when injected *in vivo* [13, 18, 22] could further enhance their capacity to elicit a protective antibody-based immunity, since DCs are powerful stimulators of CD4 T cells, which are needed for the correct development of a B-cell based immune response. Furthermore, in a vaccination scenario, the stable expression of the antigen from transduced cells might preclude the need for several injections.

Indeed, it is widely accepted that the humoral immune response is the essential component of protective immunity against West Nile Virus (WNV) [23-25]. The envelope E-glycoprotein from WNV, which possesses neutralizing epitopes [26], elicits protective immune responses when injected as a

recombinant antigen [27] or expressed either by naked DNA [28] or a replicative measles vector [29]. The fact that passive transfer of neutralizing antibodies to the soluble form of the envelope E glycoprotein (sE) from WNV strain IS-98-ST1 protected mice from WNV encephalitis further demonstrates that the humoral response is sufficient for protection against WNV challenge [29].

WNV is a mosquito-borne flavivirus in the Japanese encephalitis serocomplex of the *Flaviviridae* family. It is transmitted in natural cycles between birds and mosquitoes, but it can infect many species of mammals [30]. Zoonotic WNV recently became a major health concern in North America, the Middle East, and Europe, due to the emergence of a more virulent strain in Israel in 1998 and then in New York in 1999 (Isr98/NY99). Severe clinical manifestations of WNV infection essentially involve the central nervous system and can cause significant mortality in humans and in a large range of animal species, particularly horses and birds (for review see [30]). As a consequence, an urgent demand exists for the development of an efficient vaccine that can provide quick, strong and long lasting immunity.

SUMMARY OF THE INVENTION

This invention aids in fulfilling these needs in the art. This invention relates to the potential of lentiviral vectors to elicit a humoral immune response. Whether immunization with a lentiviral-based vaccine could protect against West Nile Virus (WNV) infection was investigated. It was surprisingly discovered that lentiviral vectors can elicit antibody based protective immunity.

In the present invention, the potential of a lentiviral vector-based vaccine was evaluated to elicit humoral immunity against West Nile Virus (WNV), a mosquito-borne flavivirus that causes encephalitis in humans, birds and horses. Remarkably, a single immunization with a minute dose of TRIP/sE_{WNV}, a lentiviral vector expressing the secreted soluble form of the envelope E-glycoprotein (sE_{WNV}) from the highly virulent IS-98-ST1 strain of WNV, induced a specific

humoral response and protection against WNV infection in a mouse model of WNV encephalitis. This single immunization elicited a long-lasting, protective and sterilizing humoral immunity, only one week after priming. These results demonstrate the applicability of lentiviral vectors as efficient non-replicating vaccines against pathogens for which a neutralizing humoral response is actively required for protective immunity. TRIP/sE_{WNV} lentiviral vector appears as a promising tool for veterinary vaccination against zoonotic WNV.

Thus, this invention provides a method of producing antibodies *in vivo* comprising administration of a lentiviral vector to an animal, wherein the lentivirus comprises a heterologous nucleic acid encoding an antigen, and wherein the antigen elicits a humoral response in the animal. The invention encompasses a lentiviral vector that induces protective immunity to an animal in need thereof, including, for example, long-lasting protective immunity and sterilizing immunity. In one embodiment, the animal in need thereof is selected from humans; horses; birds; poultry; pigs; cattle, including bovines, ovins, and caprins; rodents, including hamsters, rats, and mice; pets; and reptiles.

This invention additionally provides a method of producing antibodies *in vivo* comprising administration of a lentiviral vector to an animal, wherein the lentivirus comprises a heterologous nucleic acid encoding an antigen, and wherein the antigen elicits a humoral response in the animal, wherein the lentiviral vector induces protective immunity against an infectious microorganism In one embodiment, the infectious microorganism is West Nile Virus. In another embodiment, the lentiviral vector comprises a nucleic acid encoding a peptide or polypeptide bearing at least a B epitope. The polypeptide bearing at least a B epitope can be, for example, a microorganism membrane protein or a fragment thereof, and in particular envelope E-glycoprotein from West Nile Virus, or a fragment thereof. The lentiviral vector may comprise a carboxyl terminal-truncated E glycoprotein from West Nile Virus lacking the transmembrane anchoring region, for example, a carboxyl terminal-truncated E glycoprotein comprising residues 1-441 of the envelope E-glycoprotein from West

Nile Virus strain IS-98-ST1. In another embodiment, the heterologous nucleic acid encodes a variant of envelope E-glycoprotein from West Nile Virus. The invention encompasses a variant heterologous nucleic acid that hybridizes under conditions of stringent hybridization, which are described hereinafter, to a nucleic acid from West Nile Virus strain IS-98-ST1 encoding envelope E-glycoprotein.

The invention also provides for a method for vaccinating an animal against West Nile Virus infection, comprising administering to an animal in need thereof, one or more times, a lentiviral vector comprising a nucleic acid encoding a peptide bearing at least a B epitope. The peptide bearing at least a B epitope can be, for example, a microorganism membrane protein or a fragment thereof, and in particular envelope E-glycoprotein from West Nile Virus, or a fragment thereof, with an acceptable physiological carrier and/or an adjuvant. *In vivo* suitable routes of administration include, for example, an intraperitoneal route, intravenous route, intramuscular route, oral route, mucosal route, sublingual route, intranasal route, subcutaneous route or intradermic route. *Ex vivo* suitable routes include administration of autologous cells transduced with the lentiviral vector [i.e. Antigen Presenting Cells (APC) as dendritic cells (DC) or B cells]. In one embodiment, the heterologous nucleic acid encodes a peptide or polypeptide bearing at least a B epitope. In a preferred embodiment, the polypeptide encodes a membrane protein or a fragment thereof. In another preferred embodiment, the heterologous nucleic acid encodes carboxyl terminal-truncated E glycoprotein from West Nile Virus lacking the transmembrane anchoring region. In another, the carboxyl terminal-truncated E glycoprotein comprises residues 1-441 of the envelope E-glycoprotein from West Nile Virus strain IS-98-ST1. A preferred embodiment of this invention encompasses administering the lentiviral vector in a limited number of doses, such as, for example, a single dose or two or three doses. In one embodiment, this dose comprises, for example, vector particles equivalent to 0.5 ng to 5000 ng of p24 antigen. In mice, the preferred dose can vary from 0.5 ng to 50 ng, while in horses the preferred dose can vary from 50 ng to 500 ng. The invention

encompasses the treatment of such animals, for example, as humans; horses; birds; poultry; pigs; cattle, including bovines, ovins, and caprins; rodents, including hamsters, rats, and mice; pets; and reptiles.

The invention also provides for a method for vaccinating an animal against microorganism infection, comprising administering to an animal in need thereof, one or more times, a lentiviral vector comprising a heterologous nucleic acid encoding an immunogenic membrane protein or a fragment thereof of such microorganism. As used herein, the term microorganism encompasses viruses, bacteria, and parasites.

In a preferred embodiment the invention provides a method for vaccinating an animal in need thereof against virus infection, such as a flavivirus infection, and in particular a method for vaccinating an animal in need thereof against West Nile Virus infection by administering a lentiviral vector comprising a variant of envelope E-glycoprotein from West Nile Virus, or a fragment thereof, with an acceptable physiological carrier and/or an adjuvant. The invention encompasses such a method of vaccination wherein the heterologous nucleic acid hybridizes under conditions of stringent hybridization to a nucleic acid from West Nile Virus strain IS-98-ST1 encoding envelope E-glycoprotein. In one embodiment, the lentiviral vector is administered one time, for example, in a dose of vector particles equivalent to 0.5 ng to 5000 ng of p24 antigen. The animal in need thereof can include, for example, humans; horses; birds; poultry; pigs; cattle, including bovines, ovines, and caprines; rodents, including hamsters, rats, and mice; pets, such as cats and dogs; and reptiles.

The invention also provides for an immunogenic composition comprising a lentiviral vector, wherein the lentiviral vector comprises a heterologous nucleic acid encoding an antigen in an amount sufficient to induce an immunogenic or protective response *in vivo*, and a pharmaceutically acceptable carrier therefor. The invention encompasses an immunogenic composition, wherein the heterologous nucleic acid encodes a peptide bearing at least a B epitope. A peptide bearing at least a B epitope can be, for example, a

microorganism membrane protein or a fragment thereof, and in particular envelope E-glycoprotein from West Nile Virus, or a fragment thereof, or a carboxyl terminal-truncated E-glycoprotein from West Nile Virus lacking the transmembrane anchoring region. In one embodiment, the carboxyl terminal-truncated E glycoprotein comprises residues 1-441 of the envelope E-glycoprotein from West Nile Virus strain IS-98-ST1.

The invention also provides for an immunogenic composition comprising a lentiviral vector, wherein the lentiviral vector comprises a heterologous nucleic acid encoding an antigen in an amount sufficient to induce an immunogenic or protective response *in vivo*, and a pharmaceutically acceptable carrier therefor, and wherein the heterologous nucleic acid encodes a variant of envelope E-glycoprotein from West Nile Virus. In one example, the heterologous nucleic acid hybridizes under conditions of stringent hybridization to a nucleic acid from West Nile Virus strain IS-98-ST1 encoding envelope E-glycoprotein.

The invention encompasses a lentiviral vector that directs the expression of a heterologous nucleic acid, wherein the vector comprises cytomegalovirus immediate early promoter, and wherein the heterologous nucleic acid encodes envelope E-glycoprotein from West Nile Virus, or a fragment thereof. The heterologous nucleic acid can encode, for example, carboxyl terminal-truncated E-glycoprotein from West Nile Virus lacking the transmembrane anchoring region. In one embodiment, the carboxyl terminal-truncated E-glycoprotein comprises residues 1-441 of envelope E-glycoprotein from West Nile Virus strain IS-98-ST1. The invention also provides for a substantially purified host cell transfected or transduced with such a lentiviral vector, and for a method for the production of envelope E-glycoprotein polypeptide from West Nile Virus, or a fragment thereof, comprising culturing a host cell transfected or transduced with such a lentiviral vector under conditions promoting expression, and recovering the polypeptide from the host cell or the culture medium.

The invention further provides for a lentiviral vector that directs the expression of a heterologous nucleic acid, wherein the vector comprises cytomegalovirus immediate early promoter, and wherein the heterologous nucleic acid encodes a variant of envelope E-glycoprotein from West Nile Virus. In one embodiment, the heterologous nucleic acid hybridizes under conditions of stringent hybridization to a nucleic acid from West Nile Virus strain IS-98-ST1 encoding envelope E-glycoprotein. The invention encompasses a substantially purified host cell transfected or transduced with such a lentiviral vector, and for a method for the production of a variant of envelope E-glycoprotein from West Nile Virus comprising culturing a host cell transfected or transduced with such a lentiviral vector under conditions promoting expression, and recovering the polypeptide from the host cell or the culture medium.

The invention further provides for a lentiviral vector that directs the expression of a heterologous nucleic acid, wherein the vector comprises cytomegalovirus immediate early promoter, and wherein the heterologous nucleic acid comprises green fluorescent protein. The invention also encompasses a substantially purified host cell transfected or transduced with this lentiviral vector.

The invention encompasses a method of vaccinating against a pathogenic agent comprising administering a lentiviral vector to an animal in need thereof, wherein the lentiviral vector comprises a heterologous nucleic acid encoding an antigen, and wherein the antigen elicits antibodies against the pathogenic agent. The invention encompasses, for example, a method of vaccinating wherein administration of the lentiviral vector elicits long-lasting humoral immunity. The invention also encompasses, for example, a method of vaccinating wherein administration of the lentiviral vector elicits neutralizing antibodies against the pathogenic agent. The animal in need thereof can include, for example, humans; horses; birds; poultry; pigs; cattle, including bovines, ovines, and caprines; rodents, including hamsters, rats, and mice; pets, such as cats and dogs; and reptiles. In one embodiment, the lentiviral vector comprises a nucleic acid encoding envelope E-glycoprotein from West Nile Virus, or a

fragment thereof, and the pathogenic agent is West Nile Virus. In another embodiment, the envelope E-glycoprotein from West Nile Virus lacks a transmembrane anchoring region. In another, the administration of lentiviral vector comprises at least one dose of vector particles equivalent to 0.5 ng to 5000 ng of p24 antigen.

The invention also provides for a method of delivering DNA for producing antibodies *in vivo* comprising administration of a lentiviral vector to an animal, wherein the lentiviral vector comprises a heterologous nucleic acid encoding an antigen, and wherein the antigen elicits a humoral response in the animal. The invention encompasses a lentiviral vector that induces protective humoral immunity to an animal in need thereof. In one embodiment, the animal in need thereof is selected from humans; horses; birds; poultry; pigs; cattle, including bovines, ovins, and caprins; rodents, including hamsters, rats, and mice; pets; and reptiles.

This invention additionally provides for a method of delivering DNA for producing antibodies *in vivo* comprising administration of a lentiviral vector to an animal, wherein the lentiviral vector comprises a heterologous nucleic acid encoding an antigen, and wherein the antigen elicits a humoral response in the animal, wherein the lentiviral vector induces protective immunity against West Nile Virus. In one embodiment, the lentiviral vector may comprise a nucleic acid encoding an envelope E-glycoprotein from West Nile Virus, or a fragment thereof. The lentiviral vector may comprise a nucleic acid encoding a carboxyl terminal-truncated E glycoprotein from West Nile Virus lacking the transmembrane anchoring region, for example, a carboxyl terminal-truncated E glycoprotein comprising residues 1-441 of the envelope E-glycoprotein from West Nile Virus strain IS-98-ST1. In another embodiment, the heterologous nucleic acid encodes a variant of envelope E-glycoprotein from West Nile Virus. The invention encompasses a variant heterologous nucleic acid that hybridizes under conditions of stringent hybridization, which are described hereinafter, to a nucleic acid from West Nile Virus strain IS-98-ST1 encoding envelope E-glycoprotein.

The invention also encompasses a method comprising administration of a lentiviral vector to an animal, wherein the lentiviral vector comprises a heterologous nucleic acid encoding an antigen, and wherein the antigen elicits a humoral response in the animal. The invention encompasses a lentiviral vector that induces protective humoral immunity to an animal in need thereof. In one embodiment, the animal in need thereof is selected from humans; horses; birds; poultry; pigs; cattle, including bovines, ovins, and caprins; rodents, including hamsters, rats, and mice; pets; and reptiles.

The invention additionally provides for a method comprising administration of a lentiviral vector to an animal, wherein the lentiviral vector comprises a heterologous nucleic acid encoding an antigen, wherein the antigen elicits a humoral response in the animal, and wherein the antigen expressed in the animal after their transduction with the lentiviral vector induces protective immunity against an infectious microorganism, such as, for example, West Nile Virus. In one embodiment, the lentiviral vector comprises a nucleic acid encoding a membrane protein from an infectious microorganism (in particular encoding an envelop protein and more preferentially, the envelope E-glycoprotein from West Nile Virus, or a fragment thereof. The lentiviral vector may comprise a nucleic acid encoding the carboxyl terminal-truncated E glycoprotein from West Nile Virus lacking the transmembrane anchoring region, for example, a carboxyl terminal-truncated E glycoprotein comprising residues 1-441 of the envelope E-glycoprotein from West Nile Virus strain IS-98-ST1. In another embodiment, the heterologous nucleic acid encodes a variant of envelope E-glycoprotein from West Nile Virus. In one embodiment, the invention encompasses a heterologous nucleic acid that hybridizes under conditions of stringent hybridization to those described hereinafter, for example, to a nucleic acid from West Nile Virus strain IS-98-ST1 encoding envelope E-glycoprotein.

Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and

advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the Expression of sE_{WNV} in 293T cells transduced with TRIP/sE_{WNV}. (A) Schematic representation of the TRIP/sE_{WNV} vector. The IS-98-ST1 cDNA coding for sE_{WNV} was subcloned from the TOPO/sE_{WNV} plasmid [29] into the TRIP lentiviral vector between the BsiW1 and BssHII cloning sites and under the control of the human cytomegalovirus immediate early promoter (CMVie). LTR = Long Terminal Repeat. (B) Detection of sE_{WNV} protein in TRIP/sE_{WNV} transduced 293T cells. 293T cells were transduced with the amount of TRIP/sE_{WNV} vector particles equivalent of 100 ng/ml of p24 antigen (left), or left untransduced (right). At 48 hours post-transduction, 293T cells were immunostained with an anti-WNV HMAF (Hyperimmune Mouse Ascitic Fluid).

Figure 2 shows the detection of anti-E_{WNV} antibodies in sera from TRIP/sE_{WNV} vaccinated mice. Vero cells were infected with WNV strain IS-98-ST1 (WNV) or mock-infected (No virus). Radio-labeled cell lysates were immunoprecipitated with pooled immune sera (dilution 1:100). Samples were analyzed by SDS-15% PAGE under non-reducing conditions. (A) Sera from TRIP/sE_{WNV} immunized mice collected at day 6, 13, 20 and 27 post-immunization (p.i). (B) Sera from resistant congenic BALB/c-MBT mice inoculated with WNV (sera to WNV). Antisera to lymphochoriomeningitis virus (LCMV) were used as a negative control. WNV structural glycoproteins C, prM and E and non-structural proteins NS3 and NS2A are shown. (C) Sera from TRIP/sE_{WNV} immunized mice collected 21 days after WNV challenge, which was performed 7 or 14 days post-immunization. As a positive control, viral antigens were immunoprecipitated with anti-WNV HMAF.

Figure 3 shows that heat-inactivation of TRIP/sE_{WNV} vector particles abolishes transduction. 293T cells were transduced with a TRIP/GFP

vector (66 ng of p24 antigen per ml), heat-inactivated (70°C for 10 min) or not. At 48 h post-transduction, GFP expression was detected by FACS.

Figure 4 shows the nucleic acid sequence (SEQ ID NO:9) of the secreted form of the West Nile Virus E-glycoprotein from strain IS-98-ST1, with start and stop codons added at the ends of the sequence.

Figure 5 shows the nucleic acid sequence (SEQ ID NO:10) of the secreted form of the West Nile Virus E-glycoprotein from strain IS-98-ST1.

Figure 6 shows the amino acid sequence (SEQ ID NO:11) of the secreted form of the West Nile Virus E-glycoprotein from strain IS-98-ST1. The signal sequence at the N-terminus is underlined.

Figure 7 shows the amino acid sequence of Domain III (SEQ ID NO:12) from West Nile Virus E-glycoprotein from strain IS-98-ST1.

Figure 8 shows the complete nucleic acid sequence of the lentiviral vector pTRIPsE_{WNV} (SEQ ID NO:13) bearing the heterologous sequence of truncated West Nile Virus E-glycoprotein from West Nile Virus strain IS-98-ST1.

DETAILED DESCRIPTION

Lentiviral vectors for inducing protective humoral responses have been discovered. In one embodiment, the lentiviral vectors are replication-defective retroviral vectors comprising cis-active sequences necessary for reverse transcription (PPT 3', PBS and R regions of the LTRs), nuclear import (cPPT-CTS), and integration (psi), and a heterologous nucleic acid encoding an antigen. In this embodiment, structural and enzymatic proteins are provided in "trans." The lentiviral vectors can comprise integration repeat (IR) sequences and Tips of the LTR, as well as transcriptional sequences as ORF and promoters. The lentiviral vector particle can be pseudotyped by any viral or non-viral envelope to facilitate its entry into the target cell. One preferred site on the lentiviral vector for insertion of the heterologous nucleic acid is between the two

LTRs, as explained below. Alternately, the heterologous nucleic acid can be, for example, inserted into the U3 or U5 regions of the LTRs, or provided as a replacement for the U3 or U5 regions of the LTRs.

In one embodiment, the lentiviral vector comprises a heterologous nucleic acid encoding a peptide bearing at least one B epitope. In preferred embodiments, the heterologous nucleic acid encodes a membrane protein from an infectious microorganism, such as, for example, an envelope protein. In one preferred embodiment, the heterologous nucleic acid encodes envelope E-glycoprotein from West Nile Virus, or a fragment thereof.

The term "lentiviral vector" means that the vector contains a polynucleotide sequence that: (1) is not associated with all or a portion of a polynucleotide with which it is associated in nature, (2) is linked to a polynucleotide other than that to which it is linked in nature, or (3) does not occur in nature. Lentiviral vectors of the invention encompass vectors derived from, for example, HIV-1, HIV-2, SIV (simian immunodeficiency virus), EIAV (equine infectious anaemia virus), FIV (feline immunodeficiency virus), CAEV (Caprine arthritis encephalitis virus), and VMV (Visna/maedi virus). Lentiviral vectors also encompass chimeric lentiviruses derived from at least two different lentiviruses.

The terms "polypeptide" and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, and includes chemically or biochemically modified or derivatized amino acids, as well as polypeptides having modified peptide backbones. The term includes fusion proteins, such as GST fusion proteins and pegylated proteins, fusion proteins with a heterologous amino acid sequence, fusions with heterologous or homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; and the like.

A "fragment" of a polypeptide sequence refers to a polypeptide sequence that is shorter than the reference sequence but that retains a biological function or activity which is recognized to be the same as the reference polypeptide. Such an activity may include, for example, the ability to stimulate an

immune response. A fragment retains at least one epitope of the reference polypeptide.

The term "purified" as used herein, means that a polypeptide that is essentially free of association with other proteins or polypeptides, for example, as a purification product of recombinant host cell culture or as a purified product from a non-recombinant source. The term "substantially purified", as used herein, refers to a mixture that contains a polypeptide and is essentially free of association with other proteins or polypeptides, but for the presence of known proteins that can be removed using a specific antibody, and which substantially purified polypeptides can be used as an antigen.

The term "antigen" as used herein, means a substance capable of stimulating an immune response. Preferred antigens encompass at least one B-epitope, wherein a B-epitope is capable of eliciting a humoral immune response. Such preferred antigens include, for example, surface antigens, such as envelope or other membrane proteins, and fragments thereof.

The term "pathogen" as used herein, means a specific causative agent of disease, and may include, for example, any bacteria, virus or parasite.

The term "disease" as used herein, means an interruption, cessation, or disorder of body function, system, or organ. Preferred diseases include infectious diseases.

The term "humoral immunity" as used herein, means antibodies elicited by an antigen, and all the accessory processes that accompany it.

The term "protective humoral immunity" as used herein, means a humoral immune response that confers the essential component of protection against a pathogen.

The term "sterilizing humoral immunity" as used herein, means a humoral immune response that prevents the establishment of any detectable infection by a pathogen.

The term "long-lasting humoral immunity" as used herein, means that some aspect of humoral immunity is detectable three months after antigen

administration, such as, for example, antibodies elicited by the antigen. Suitable methods of antibody detection include, but are not limited to, such methods as ELISA, immunofluorescence (IFA), focus reduction neutralization tests (FRNT), immunoprecipitation, and Western blotting.

The term "neutralizing humoral response" as used herein, means that the antibodies elicited during humoral immunity directly block the ability of a pathogen to infect cells.

The term "quick response" as used herein, means that protective humoral immunity is conferred within three weeks of antigen administration. The term "very quick response" as used herein, means that protective humoral immunity is conferred within one week of antigen administration.

Hybridization reactions can be performed under conditions of different "stringency." Conditions that increase stringency of a hybridization reaction are known in the art. For example, stringent conditions for both DNA/DNA and DNA/RNA hybridization are described in Sambrook and Russell, *Molecular Cloning, A Laboratory Manual*, 3rd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001. Moreover, a person skilled in the art would know how to modify the conditions as necessary for the degree of stringency required for a particular hybridization.

Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25°C, 37°C, 50°C and 68°C; buffer concentrations of 10 x SSC, 6 x SSC, 1 x SSC, 0.1 x SSC (where 1 x SSC is 0.15 M NaCl and 15 mM citrate buffer) and their equivalents using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 minutes to 24 hours; 1, 2, or more washing steps; wash incubation times of 1, 2, or 15 minutes; and wash solutions of 6 x SSC, 1 x SSC, 0.1 x SSC, or deionized water.

An example of stringent hybridization conditions is hybridization at 50°C and 0.1×SSC (15 mM sodium chloride/1.5 mM sodium citrate). Another example of stringent hybridization conditions is overnight incubation at 42°C in a

solution containing 50% formamide, 1 × SSC (150 mM NaCl, 15 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5 × Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1 × SSC at about 65°C. A further example of high stringency conditions includes aqueous hybridization (e.g., free of formamide) in 6X SSC (where 20X SSC contains 3.0 M NaCl and 0.3 M sodium citrate), 1% sodium dodecyl sulfate (SDS) at 65°C for about 8 hours (or more), followed by one or more washes in 0.2 X SSC, 0.1% SDS at 65°C.

A "variant" polypeptide as referred to herein means a polypeptide substantially homologous to the reference polypeptide, but which has an amino acid sequence different from that of reference polypeptides because of one or more deletions, insertions, or substitutions. The variant amino acid sequence preferably is at least 90% identical to the reference polypeptide, and most preferably at least 95% identical. The percent identity can be determined, for example by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (*J. Mol. Biol.* 48:443, 1970), as revised by Smith and Waterman (*Adv. Appl. Math* 2:482, 1981). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

Variants can comprise conservatively substituted sequences, meaning that a given amino acid residue is replaced by a residue having similar physiochemical characteristics. Examples of conservative substitutions include

substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known. Naturally occurring polypeptide variants are also encompassed by the invention. Examples of such variants are proteins that result from alternate mRNA splicing events or from proteolytic cleavage of the polypeptides. Variations attributable to proteolysis include, for example, differences in the termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the polypeptides. Variations attributable to frameshifting include, for example, differences in the termini upon expression in different types of host cells due to different amino acids.

The term "binds specifically," in the context of antibody binding, refers to high avidity and/or high affinity binding of an antibody to a specific polypeptide, or more accurately, to a specific epitope of a specific polypeptide. Antibody binding to such epitope is typically stronger than binding of the same antibody to any other epitope or any other polypeptide which does not comprise the epitope. Such an antibody is typically produced by injecting the specific polypeptide into an animal to elicit the production of antibodies. Such an antibody may be capable of binding other polypeptides at a weak, yet detectable, level (e.g., 10% or less of the binding shown to the polypeptide of interest). Such weak binding, or background binding, is readily discernible from the specific antibody binding, for example, by use of appropriate controls. In general, antibodies of the invention specifically bind to a specific polypeptide with a binding affinity of 10^{-7} M or more, preferably 10^{-8} M or more (e.g., 10^{-9} M, 10^{-10}, 10^{-11}, etc.).

A "pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any conventional type. A "pharmaceutically acceptable carrier" is

non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the carrier for a formulation containing polypeptides preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides. Suitable carriers include, but are not limited to, water, dextrose, glycerol, saline, ethanol, and combinations thereof. The carrier may contain additional agents such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the formulation. Topical carriers include liquid petroleum, isopropyl palmitate, polyethylene glycol, ethanol (95%), polyoxyethylene monolaurate (5%) in water, or sodium lauryl sulfate (5%) in water. Other materials such as anti-oxidants, humectants, viscosity stabilizers, and similar agents may be added as necessary. Percutaneous penetration enhancers such as Azone may also be included.

The complete genome sequence of West Nile Virus strain IS-98-ST1 (or STD) is available on GenBank accession number AF 481864 (GI: 19387527, version of May 21, 2002).

The invention provides for isolated and purified, or homogeneous, envelope E-glycoprotein polypeptides from West Nile Virus, both recombinant and non-recombinant. Variants and derivatives of native envelope E-glycoprotein polypeptides that can be used as antigens can be obtained by mutations of nucleotide sequences coding for native envelope E-glycoprotein polypeptides. Alterations of the native amino acid sequence can be accomplished by any of a number of conventional methods. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene wherein predetermined codons can be altered by substitution, deletion, or insertion. Exemplary methods

of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); Kunkel (*Proc. Natl. Acad. Sci. USA* 82:488, 1985); Kunkel et al. (*Methods in Enzymol.* 154:367, 1987); and U.S. Patent Nos. 4,518,584 and 4,737,462, all of which are incorporated by reference.

Within an aspect of the invention, the lentiviral vector can be utilized to prepare antibodies that specifically bind to polypeptides. The term "antibodies" is meant to include polyclonal antibodies, monoclonal antibodies, fragments thereof such as F(ab')2 and Fab fragments, as well as any recombinantly produced binding partners. Antibodies are defined to be specifically binding if they bind envelope E-glycoprotein polypeptides with a K_a of greater than or equal to about 10^7 M^{-1}. Affinities of binding partners or antibodies can be readily determined using conventional techniques, for example, those described by Scatchard et al., *Ann. N.Y Acad. Sci.*, *51*:660 (1949). Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice, or rats, using procedures that are well known in the art.

Recombinant expression vectors containing can be prepared using well known methods. For a review of molecular biology techniques see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, CSHL Press 2001, 3rd Ed.. The expression vectors can include an insert sequence, such as West Nile Virus envelope E-glycoprotein sequence, operably linked to suitable transcriptional or translational regulatory nucleotide sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, an mRNA ribosomal binding site, and appropriate sequences which control transcription and translation initiation and termination. Nucleotide sequences are "operably linked" when the regulatory sequence functionally relates to the insert sequence. Thus, a promoter nucleotide sequence is operably linked to a

envelope E-glycoprotein sequence if the promoter nucleotide sequence controls the transcription of the envelope E-glycoprotein DNA sequence. The ability to replicate in the desired host cells, usually conferred by an origin of replication, and a selection gene by which transformants are identified can additionally be incorporated into the expression vector.

In addition, sequences encoding appropriate signal peptides that are not naturally associated with the insert sequence can be incorporated into expression vectors.

Suitable host cells for expression include prokaryotes, yeast or higher eukaryotic cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described, for example, in Pouwels et al. *Cloning Vectors: A Laboratory Manual,* Elsevier, New York, (1985).

Once the lentiviral vector of the invention have been obtained, they can be used to produce polyclonal and monoclonal antibodies. Thus, a protein or polypeptide expressed *in vivo* or *ex vivo* by means of the lentiviral vector of the invention can be used to immunize an animal host by techniques known in the art. Such techniques usually involve inoculation, but they may involve other modes of administration. A sufficient amount of the lentiviral vector is administered to create an immunogenic response in the animal host. Any host that can be infected by lentivirus can be used. Once the animal has been immunized and sufficient time has passed for it to begin producing antibodies to the antigen, polyclonal antibodies can be recovered. The general method comprises removing blood from the animal and separating the serum from the blood. The serum, which contains antibodies to the antigen, can be used as an antiserum to the antigen. Alternatively, the antibodies can be recovered from the serum. Affinity purification is a preferred technique for recovering purified polyclonal antibodies to the antigen, from the serum.

Monoclonal antibodies to the antigens of the invention can also be prepared. One method for producing monoclonal antibodies reactive with the

antigens comprises the steps of infecting the host with the lentiviral vector; recovering antibody producing cells from the spleen of the host; fusing the antibody producing cells with myeloma cells deficient in the enzyme hypoxanthine-guanine phosphoribosyl transferase to form hybridomas; selecting at least one of the hybridomas by growth in a medium comprising hypoxanthine, aminopterin, and thymidine; identifying at least one of the hybridomas that produces an antibody to the antigen, culturing the identified hybridoma to produce antibody in a recoverable quantity; and recovering the antibodies produced by the cultured hybridoma.

These polyclonal or monoclonal antibodies can be used in a variety of applications. Among these is the neutralization of corresponding proteins. They can also be used to detect viral antigens in biological preparations or in purifying corresponding proteins, glycoproteins, or mixtures thereof, for example when used in an affinity chromatographic columns.

The envelope E-glycoprotein polypeptides can be used as antigens to identify antibodies to West Nile Virus in materials and to determine the concentration of the antibodies in those materials. Thus, the antigens can be used for qualitative or quantitative determination of the virus in a material. Such materials include animal tissue and animal cells, as well as biological fluids, such as animal body fluids, including animal sera. When used as a reagent in an immunoassay for determining the presence or concentration of the antibodies to West Nile Virus, the antigens provide an assay that is convenient, rapid, sensitive, and specific.

More particularly, the antigens can be employed for the detection of microorganism pathogen infection (in particular West Nile Virus) by means of immunoassays that are well known for use in detecting or quantifying humoral components in fluids. Thus, antigen-antibody interactions can be directly observed or determined by secondary reactions, such as precipitation or agglutination. In addition, immunoelectrophoresis techniques can also be employed. For example, the classic combination of electrophoresis in agar

followed by reaction with anti-serum can be utilized, as well as two-dimensional electrophoresis, rocket electrophoresis, and immunolabeling of polyacrylamide gel patterns (Western Blot or immunoblot). Other immunoassays in which the antigens of the present invention can be employed include, but are not limited to, radioimmunoassay, competitive immunoprecipitation assay, enzyme immunoassay, and immunofluorescence assay. It will be understood that turbidimetric, colorimetric, and nephelometric techniques can be employed. An immunoassay based on Western Blot technique is preferred.

Immunoassays can be carried out by immobilizing one of the immunoreagents on a carrier surface while retaining immunoreactivity of the reagent. The reciprocal immunoreagent can be unlabeled or labeled in such a manner that immunoreactivity is also retained. These techniques are especially suitable for use in enzyme immunoassays, such as enzyme linked immunosorbent assay (ELISA) and competitive inhibition enzyme immunoassay (CIEIA).

When either of the immunoreagents are attached to a solid support, the support is usually a glass or plastic material. Plastic materials molded in the form of plates, tubes, beads, or disks are preferred. Examples of suitable plastic materials are polystyrene and polyvinyl chloride. If the immunoreagent does not readily bind to the solid support, a carrier material can be interposed between the reagent and the support. Examples of suitable carrier materials are proteins, such as bovine serum albumin, or chemical reagents, such as glutaraldehyde or urea. Coating of the solid phase can be carried out using conventional techniques.

The invention provides immunogenic lentiviral vector, and more particularly, protective lentiviral vector for eliciting a protective response against West Nile Virus. These lentiviral vectors can thus be employed as viral vaccines by administering the lentiviral vector to an animal susceptible to a pathogenic microorganism (a bacteria, a virus, a parasite) and are useful in preventing infection. In one embodiment, the lentiviral vectors of the invention comprise a

heterologous nucleic acid encoding a membrane protein from flavivirus, for example, from West Nile Virus, and are useful to prevent infections, for example, from West Nile Virus infection. Conventional modes of administration can be employed. For example, administration can be carried out by oral, respiratory, parenteral routes, sublingual route, intranasal route, subcutaneous route or intradermic route.

The vaccine compositions of the invention are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated including, e.g., the capacity of the individual's immune system to induce an immune response.

The immunization schedule will depend upon several factors, such as the susceptibility of the host to infection, the weight of the host, and the age of the host. A single dose of the vaccine of the invention can be administered to the host or a primary course of immunization can be followed in which several doses at intervals of time are administered. Subsequent doses used as boosters can be administered as needed following the primary course.

An immunogenic response can be obtained by administering the lentiviral vector of the invention to the host in an amount of about 10 to about 500 micrograms antigen per kilogram of body weight, preferably about 50 to about 100 micrograms antigen per kilogram of body weight. The proteins and vaccines of the invention can be administered together with a physiologically acceptable carrier. For example, a diluent, such as water or a saline solution, can be employed.

To further achieve the objects and in accordance with the purposes of the present invention, a kit capable of diagnosing a West Nile Virus infection is described. This kit, in one embodiment, contains the antibodies of this invention, which are capable of binding to West Nile Virus envelope E-glycoprotein. This kit, in another embodiment, contains the polypeptides of this invention, which are capable of detecting the presence or absence of antibodies,

which bind to the envelope E-glycoprotein polypeptide.

This invention will be described in greater detail in the following Examples.

Example 1: Materials and Methods

Cell culture and virus preparations. Human 293T cells and African green monkey kidney Vero cells were grown in Dulbecco's modified Eagle medium (DMEM) Glutamax (GIBCO) supplemented with 10% or 5% heat-inactivated Fetal Calf Serum (FCS) respectively. WNV strain IS-98-ST1 (GenBank accession number AF 481864) [31], a closely related variant of NY99 strain [32-34], was propagated in mosquito *Aedes pseudoscutellaris* AP61 cell monolayers. Purification in sucrose gradients, and virus titration on AP61 cells by focus immunodetection assay (FIA) using anti-WNV Hyperimmune Mouse Ascitic Fluid (HMAF) were performed as previously described [29, 31, 35]. Infectivity titers were expressed as Focus Forming Units (FFU).

Lentiviral vector construction and production. The 1.4 kb cDNA coding for the carboxyl terminal-truncated E glycoprotein lacking the transmembrane-anchoring region (residues E-1 to E-441; referred to as sE_{WNV}) from WNV strain IS-98-ST1 is described elsewhere [29]. The sE_{WNV} coding cDNA was already modified by PCR to be flanked on the open reading frame extremities by BsiWI and BssHII restriction endonucleases sites. The cDNA was digested with BsiWI and BssHII and then cloned into the unique BsiWI and BssHII sites of the pTRIP ΔU3 CMV plasmid. The resulting vector plasmid, pTRIP.ΔU3.CMV.sE_{WNV} (hereafter referred to as pTRIP/sE_{WNV}), contains the IS-98-ST1 sE glycoprotein sequence under the control of the cytomegalovirus immediate early promoter (CMVie).

Vector particles were produced by transient calcium phosphate co-transfection of 293T cells with the vector plasmid pTRIP/sE_{WNV}, an encapsidation plasmid (p8.7, [36]) and a VSV-G envelope expression plasmid

(pHCMV-G; [37]) as previously described [38]. Quantification of p24 antigen content of concentrated vector particles was done with a commercial HIV-1 p24 ELISA kit (Perkin Elmer LifeSciences).

Quantitative PCR. For detection of the U5-R sequences in the lentiviral vector, primers and probes used were as follows: probes LTR-FL 5'-CACAACAGACGGGCACACACTACTTGA-fluorescein-3' (SEQ ID NO:1), LTR-LC 5'-RED640-CACTCAAGGCAAGCTTTATTGAGGC-phosphorylated-3' (SEQ ID NO:2), primers AA55M 5'-GCTAGAGATTTTCCACACTGACTAA-3' (SEQ ID NO:3), M667 5'-GGCTAACTAGGGAACCCACTG-3' (SEQ ID NO:4)[39]. For detection of CD3, the sequences of primers and probes were as follows: Probes CD3-P1 5'-GGCTGAAGGTTAGGGATACCAATATTCCTGTCTC-fluorescein-3' (SEQ ID NO:5), CD3-P2 5'-RED705-CTAGTGATGGGCTCTTCCCTTGAGCCCTTC-phosphorylated-3' (SEQ ID NO:6) and primers CD3-in-F 5'-GGCTATCATTCTTCTTCAAGGTA-3' (SEQ ID NO:7) and CD3-in-R 5'-CCTCTCTTCAGCCATTTAAGTA-3' (SEQ ID NO:8). Primers and probes were synthesized by Proligo (France). Genomic DNA from approximately 3.10^6 lentiviral vector transduced 293T cells was isolated 48h after transduction using QIAamp® DNA Blood Mini Kit (QIAGEN, GmbH, Hilden). For real-time PCR analysis, 5μL of DNA were mixed with 15μL of a PCR master mix consisting of 1X Jumpstart™ Taq ReadyMix™ (Sigma), 1.9mM $MgCl_2$, 1.5μM of forward and reverse primers (AA55M/M667 or CD3-in-F/CD3-in-R), 200nM of the probes (LTR-FL/LTR-LC or CD3-P1/CD3-P2) and, 1.5 units of Taq DNA Polymerase (Invitrogen). Amplifications were performed on a LightCycler 2.0 (Roche Applied Science), using one cycle of 95°C for 3 min, and 40 cycles of 95°C for 5 s, 55°C for 15 s and 72°C for 10 s. To take into account the possible plasmid contamination of vector stocks, DNA from 293T cells transduced with heat-inactivated (10 min at 70°C) vector was always tested in parallel. For negative controls 5μL of genomic DNA from untransduced cells was used. Each DNA sample was tested in duplicate and the mean values are reported. Ten-fold

serial dilutions of known concentration of the plasmid pTripCD3, containing the relevant sequences U5-R and CD3, were amplified in parallel with DNA samples to generate a standard curve.

The total number of vector copies per cell was calculated by normalizing the number of U5-R copies to the number of 293T cells, as quantified by the copy number of CD3 molecules on the same genomic DNA sample, and then subtracting the number of copies obtained for the heat-inactivated vector-transduced cells.

Mouse antisera to WNV. Anti-WNV Hyperimmune Mouse Ascitic Fluid (HMAF) was obtained by repeated immunization of adult mice with WNV strain IS-98-ST1 followed by the inoculation of sarcoma 180. The sera to WNV were obtained by immunization of adult WNV-resistant BALB/c-MBT congenic mice with 10^3 FFU of IS-98-ST1 as described previously [31]. Mouse polyclonal anti-WNV antibodies were collected 1 month after inoculation.

Mice immunization and WNV challenge. All animal experiments were conducted in accordance with the guidelines of the Office Laboratory of Animal Care at the Pasteur Institute. Six to eight-week-old 129 mice were intraperitoneally (i.p.) inoculated with varying doses of TRIP/sE_{WNV} vector particles in 0.1 ml Dulbecco's PBS (DPBS; pH 7.5) supplemented with buffered 0.2% bovine serum albumin (DPBS/0.2%BSA, Sigma). WNV challenge was performed by i.p. inoculation of neurovirulent WNV strain IS-98-ST1 (i.p. LD_{50} =10 FFU) as previously described [29, 31]. The challenged mice were monitored daily for signs of morbidity or mortality, for up to 21 days.

Measurement of serum antibody responses. Mice were bled via the periorbital route and sera were heat-inactivated for 30 min at 56 °C. Anti-WNV antibodies were detected by ELISA using sucrose-purified WNV IS-98-ST1 as viral antigen [29, 31]. Peroxidase-conjugated anti-mouse immunoglobulin (H+L) (Jackson Immuno Research) at a 1:4,000 dilution, and peroxidase-conjugated anti-mouse IgM (μ-chain specific) or IgG (γ-chain specific) (Sigma) at a 1:20,000 dilution were used as secondary antibodies. The end point titer was

calculated as the reciprocal of the last dilution eliciting twice the optical density (OD) of sera from TRIP/GFP inoculated mice that served as negative controls.

Anti-WNV neutralizing antibodies were detected by a focus reduction neutralization test (FRNT) on Vero cells as previously described [29]. The end-point titer was calculated as the reciprocal or the highest serum dilution tested that reduced the number of FFU by 90% ($FRNT_{90}$).

Radioimmunoprecipitation assay. Vero cells cultured on $25cm^2$ flask were infected with WNV strain IS-98-ST1 at a high multiplicity of infection. At 20h post-infection, cells were starved for 1 h in DMEM depleted in methionine (ICN Biomedicals) and radiolabeled with 100 µCi/ml Trans^{35}S-Label^{TM}/ml (ICN Biomedicals) for 3 h. After three washes with cold PBS, cells were lysed with RIPA lysis buffer (50 mM Tris-Cl, 150 mM NaCl, 10 mM EDTA, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, pH 8.0) supplemented with 25 µg/ml aprotinin (Sigma) for 10 min at 4°C. The cell lysates were then clarified by centrifugation at 10,000 rpm for 5 min at 4°C. Radioimmunoprecipitation (RIP) assay was performed as described previously [29, 40]. Viral antigens were immunoprecipitated with mouse anti-WNV antibodies. The samples were analyzed by SDS-15% PAGE under non-reducing conditions.

Indirect immunofluorescence and flow cytometry assays. For indirect immunofluorescence (IF) analysis, human 293T cells cultured on 8-chamber Glass-Labteks (Nunc) were transduced with TRIP/sE_{WNV} vector particles. After 48 h, cells were fixed with 3% paraformaldehyde (PFA) in PBS for 20 min and permeabilized with 0.1% Triton X-100 in PBS for 4 min. Cells were incubated with anti-WNV HMAF at a 1:100 dilution in PBS for 1h. After blocking with DPBS/0.2% BSA, cells were further incubated with a Cy3-conjugated anti-mouse IgG antibody (Amersham Pharmacia) at a 1:500 dilution in DPBS/0.2% BSA. Cell nuclei were visualized with DAPI. The slides were examined using a Zeiss Axioplan microscope with ApoTome system.

For flow cytometry analysis, 293T cells cultured on 25 cm^2 flasks were transduced with TRIP/GFP, either heat-inactivated (70°C for 10 min) or not.

At 48 h, cells were detached, washed and fixed with 2% PFA. The GFP fluorescence intensity was measured by FACS and analyzed with CellQuest software.

Example 2: Expression of the secreted form of the E glycoprotein of WNV by the recombinant TRIP lentiviral vector.

To evaluate whether immunization with a lentiviral vector-based vaccine can protect against WNV encephalitis, a lentiviral vector was generated, TRIP/sE_{WNV}, which expresses a soluble form of the E glycoprotein from the WNV strain IS-98-ST1 (sE_{WNV}), under the control of the CMV immediate early promoter ($CMV_{i.e.}$) (Fig.1A). We previously demonstrated the efficient secretion of sE_{WNV} in the culture medium of cells infected by a recombinant measles vector [29]. Expression of sE_{WNV} in lentiviral vector-transduced 293T cells was examined by immunofluorescence (Fig. 1B). At 48h post-transduction, a high fraction of cells were immunostained with a pattern suggesting that sE_{WNV} migrated through the secretory pathway.

The amount of physical particles in the vector stock used in this study was determined by a commercially available ELISA assay against the p24 HIV-1 capsid protein (Perkin Elmer Lifescience). The vector stock's actual titer was calculated on the basis of the transfer of vector DNA to target cells, using a quantitative PCR assay. The quantification of both a vector specific sequence (U5) and a cellular locus (CD3) gives the average vector DNA copy number per cell. This allows a precise titration of the vector preparation. The TRIP/sE_{WNV} vector stock used in this study was titrated in human 293T cells at 5.2 $x10^7$ transduction units (TU) per ml, (corresponding to approximately 900 TU/ng of p24 in human 293T cells). For simplicity reasons, we will express, in the following sections, the quantity of vector particles used as ng of p24 antigen.

Example 3: A single immunization with TRIP/sE_{WNV} induces strong antibody responses.

To assess the humoral immune response induced by the lentiviral vector expressing sE_{WNV}, groups of adult 129 mice were inoculated i.p with a single dose of vector particles equivalent to 500 ng of p24 antigen. Vectors coded either for sE_{WNV} or for GFP protein as a control. Mice were bled periorbitally at 6, 13, 20 or 27 days post-immunization, and individual or pooled sera were tested for anti-WNV total antibodies, IgG and IgM using an already described isotype specific ELISA [29]. *In vitro* neutralizing activity of sera was assayed by a focus reduction neutralization test (FNRT) [29].

No anti-WNV antibodies were detected in sera of TRIP/GFP immunized control mice. Sera from TRIP/sE_{WNV} immunized mice were first tested individually and very small differences between animals were observed (*Mean±SD* at day 6 = 0.5±0.3; day 13 = 1.3±0.05; day 21= 1.3±0,15; day 27 = 1.4±0.15), thus in following experiments, pooled sera were used. In TRIP/sE_{WNV} immunized mice, total antibodies against WNV were detectable as early as 6 days post immunization, although present at a low concentration. As expected at this time point, only anti-WNV IgM antibodies were detected in the immune sera. Total antibody responses increased 10-fold to reach a plateau at day 13, and were then maintained over time. At these later time points (day 13, 20, 27), IgM antibodies disappeared, to be replaced by IgG (Table 1). These sera were reactive with WNV E-glycoprotein from IS-98-ST1 infected Vero cell lysates as demonstrated by RIP assay (Fig 2A).

A focus reduction neutralization test (FRNT) showed that sera from TRIP/sE_{WNV} immunized mice contained detectable levels of neutralizing anti-WNV antibodies as early as 6 days post-immunization (Table 1). Together, these data show that an early and specific anti-WNV antibody immune response is mounted in mice inoculated with a single dose of TRIP/sE_{WNV} vector particles.

TABLE 1. Antibody response of mice to inoculation with TRIP/sE$_{WNV}$

Immunizing vector[a], Day of bleeding	WNV antibody titer[b]	WNV IgM antibody titer[b]	WNV IgG antibody titer[b]	Anti-WNV FRNT$_{90}$[c]
TRIP/GFP				
Day 27	ND	ND	ND	< 10
TRIP/sE$_{WNV}$				
Day 06	3,000	300	ND	10
Day 13	30,000	ND	1,000	10
Day 20	30,000	ND	1,000	10
Day 27	30,000	ND	1,000	20

a. Groups of 6 adult 129 mice were inoculated i.p. with a quantity of lentiviral vector particles corresponding to 500 ng of p24 antigen.

Example 4: Immunization with TRIP/sE$_{WNV}$ confers early protection of mice against WNV encephalitis.

In order to establish if the humoral immunity elicited in mice after TRIP/sE$_{WNV}$ vaccination was protective, advantage was taken of a mouse model for WNV challenge. Indeed, some mice strains are extremely sensitive to WNV challenge [31, 41], they develop a neuroinvasive lethal disease similar to that found in humans [32, 42] and die within few days post-inoculation. Groups of 129 mice that received a single dose of 500 ng p24 of TRIP/sE$_{WNV}$ or TRIP/GFP vector particles were i.p. challenged with 1,000 i.p.LD$_{50}$ of WNV strain IS-98-ST1 one or two weeks after priming.

Remarkably, all mice immunized with TRIP/sE$_{WNV}$ were protected

against a high viral challenge (1,000 i.p. LD_{50}) as early as 7 days after priming. All mice immunized with the control vector TRIP/GFP or with DPBS died within 11 days of challenge (Table 2). Interestingly, total antibodies against WNV increased by ten-fold after challenge, suggesting that an effective secondary response was mounted in TRIP/sE_{WNV} immunized mice (Table 2). Equivalent results were obtained in BALB/c mice (data not shown). These results indicate that TRIP/sE_{WNV} vaccination confers a very quick, fully protective immune response

TABLE 2. Rapid protection by TRIP/sE_{WNV} against WNV infection

Immunizing vector [a], day of challenge	Protection [b] (n° of surviving/n° of infected)	Post-challenge WNV antibody titer [c]
DPBS		
Day 7	0/2	NA
Day 14	0/2	NA
TRIP/GFP		
Day 7	0/2	NA
Day 14	0/2	NA
TRIP/sE_{WNV}		
Day 7	6/6	200,000
Day 14	6/6	300,000

a. Groups of adult 129 mice were inoculated i.p. with a single dose of lentiviral vector particles corresponding to 500 ng of p24 antigen or with DPBS.

against a high WNV challenge.

Example 5: Immunization with TRIP/sE$_{WNV}$ elicits sterilizing antiviral immunity.

To address whether or not WNV primo-infection can take place in vaccinated animals upon challenge, RIP assays were performed on pooled sera from immunized mice collected before and 21 days after WNV challenge.

The E protein was first detectable by RIP assay at day 13 post-immunization using pooled sera from TRIP/sE$_{WNV}$ immunized mice (Fig.2A). The lack of detection of antibodies to E in one-week immune sera can be attributed to the low avidity of protein A for IgM in our RIP assay. Sera from TRIP/GFP control immunized mice did not react with WNV E protein.

Importantly, post-challenge sera from TRIP/sE$_{WNV}$ vaccinated mice did not precipitate any viral protein other than the E (Fig. 2C). This lack of reactivity against any WNV proteins other than E (such as preM and non structural proteins NS2a, NS2b and NS3) clearly indicates the absence of *in vivo* WNV replication upon challenge in vaccinated animals. In the BALB/c-MBT mice strain, which is resistant to the WNV encephalitis, sera are readily reactive to all viral proteins (Fi 2B). Thus, TRIP/sE$_{WNV}$ vaccination confers full sterilizing immunity to mice.

Example 6: Protection provided by a single injection of TRIP/sE$_{WNV}$ is long lasting.

We next determined whether a single immunization with the lentiviral vector-based vaccine has the potential to elicit long-term protective immunity against WNV infection. Groups of 129 mice were i.p. immunized with TRIP/sE$_{WNV}$ vector particles equivalent to 500 ng of p24 antigen or the same amount of TRIP/GFP vector as a control. Three months later, mice were bled periorbitally and pooled sera from each group were tested by ELISA and FRNT. Antibody levels in TRIP/sE$_{WNV}$ immunized mice were still remarkably high 3

months after a single vector injection (1:30,000), and neutralizing antibodies persisted (Table 3). Mice were then challenged i.p. with a 1,000 LD_{50} dose of IS-98-ST1 WNV. Neither morbidity nor mortality were observed in mice immunized with TRIP/sE_{WNV} whereas all control mice died within 10 days (Table 3). Total antibody titers as well as neutralizing anti-WNV antibodies increased after challenge, suggesting that an effective secondary response was mounted in mice immunized with a TRIP/sE_{WNV} three months earlier (Table 3).

Thus, a single dose of the sE_{WNV} coding lentiviral vector is efficient for providing long-term protective immunity in mice.

TABLE 3. Long-term protection by TRIP/sE_{WNV} against WNV infection

Immunizing vector[a]	WNV antibody titer[b] (pre-challenge)	Anti-WNV $FRNT_{90}$[c] (pre-challenge)	Protection[d] n° of surviving/ n°of infected	WNV antibody titer[b] (post-challenge)	Anti-WNV $FRNT_{90}$[c] (post-challenge)
TRIP/GFP	ND	<10	0/3	NA	NA
TRIP/sE_{WNV}	30,000	20	13/13	500,000	400

a. Groups of adult 129 mice were immunized i.p. with lentiviral vector particles corresponding to 500 ng of p24 antigen.

b. Determined by ELISA on pooled heat-inactivated sera.

c. FRNT: Focus Reduction Neutralization Test: the highest serum dilution that reduced the number of FFU of WNV by at least 90%.

d. Mice were inoculated i.p. with 1,000 LD_{50} of WNV strain IS-98-ST1, three months post immunization. Survival was recorded for 21 days.

ND : not detected (<100)

NA : not applicable

Example 7: A single minute dose of TRIP/sE_{WNV} confers full and rapid protection.

In order to determine the minimum protective vaccine dose, groups of 129 mice were inoculated once with a wide range of TRIP/sE_{WNV} vaccine doses varying from 1.5 ng to 500 ng of p24 antigen. One week after priming, immunized mice were challenged i.p. with 1,000 LD_{50} IS-98-ST1 WNV. As expected, all mice that received 500 ng of p24 of the control TRIP/GFP vector died within 11-13 days after challenge. A 100% protection was achieved after injection of a single TRIP/sE_{WNV} vector dose as low as 50 ng of p24. Lower doses conferred only partial protection thus allowing us to calculate a 50% protective dose in adult mice of 6.2 ng of p24 antigen (Table 4). Of note, these dose-protection experiments were performed in the most stringent challenge conditions, with an early challenge at day 7 post-vaccination and with a high lethal virus challenge inoculum (1,000 LD_{50}). Since total antibody concentrations increased by ten-fold between days 7 and 14 (Table 1), it is probable that the 50% protective dose would have been even lower than 6.2 ng if determined only one week later.

These results demonstrate that a minute dose of vector particles achieves quick and fully protective immunity in mice. This 100% protection is the consequence of an actual *in vivo* cell transduction by the vector particles. A heat treatment (10 min at 70°C) of the vector particles that abrogates the transduction capability of lentiviral vectors (Fig 3) also abrogates protection (Table 4), ruling out the possibility of a protection conferred by plasmid DNA or residual sE_{WNV} proteins contaminating the vector stock.

TABLE 4. Dose-dependent protection by TRIP/sE_{WNV} against WNV infection

Immunizing vector[a], dose (ng of p24)	Protection[b] n° of surviving/ n° of infected	Post-challenge WNV antibody titer[c]
TRIP GFP		
500	0/6	NA
Heat-inactivated TRIP/sE_{WNV}[d]		
50	0/6	NA
TRIP/sE_{WNV}		
500	6/6	200,000
150	6/6	300,000
50	12/12	300,000
15	5/6	300,000
5	2/5	200,000
1.5	1/12	NA

a. Groups of adult 129 mice were inoculated i.p. with a single dose of lentiviral vector particles.

b. Mice were inoculated i.p. with 1000 LD50 of WNV strain IS-98-ST1 one week after priming. Survival was recorded for 21 days.

c. Determined by ELISA on pooled heat-inactivated sera.

d. Lentiviral vector particles were heat-inactivated for 10 min at 70°C.

NA : Not Applicable

This invention provides the first evidence that lentiviral vectors are efficient tools for eliciting a protective humoral immune response against a pathogen. In addition to the now well-described capacity of these vectors for inducing strong cellular immune responses [12-15, 17, 18, 21], this work broadens the applicability of lentiviral vectors as vaccination tools against pathogens for which a neutralizing humoral response is one active arm of the immune system.

The TRIP/sE_{WNV} vector was able to induce a very early, long-lasting, fully protective immune response against WNV regardless of the lethal dose used in mice. Importantly, this immunity was achieved by a single immunization with a minute dose of vector particles.

These features make of TRIP/sE_{WNV} a promising vaccine candidate against WNV. In particular, the urgent need for an efficient veterinary vaccine that could be used in susceptible animal populations in the event of a WNV outbreak, justifies trials involving the lentiviral TRIP/sE_{WNV} vector in animals, notably horses and birds. Furthermore, the prophylactic immunization of large groups of animals with lentiviral vector-based vaccines would also serve as solid toxicity and safety proofs of concepts, providing the necessary hindsight and eventually paving the way for the possible use of lentiviral vectors as prophylactic vaccination tools in humans.

Several lines of arguments make the TRIP/sE_{WNV} lentiviral vector a powerful candidate vaccine for veterinary use. Firstly, the provided protection takes place soon after immunization. This could be of major importance in the context of a WNV outbreak where rapid protection of sensitive animals is crucial. The exact nature of this early protective immune response has not been fully addressed. One week after immunization IgG antibodies are not yet detectable and IgM antibodies probably account for the observed protection. Passive transfer of polyclonal anti-WNV IgM has been shown to protect mice from WNV challenge, demonstrating the critical role of this isotype in controlling the early phases of WNV infection [43]. Nevertheless, we cannot exclude the contribution

of innate or adaptive cellular responses against WNV antigens to the early protection observed [44]. Secondly, the TRIP/sE_{WNV} lentiviral vector is fully effective with a single minute dose. This makes this candidate vaccine interestingly cost-effective, and could allow the development of protocols for mass vaccination, of particular interest for the protection of poultry. It is important to note that the dose required for full protective immunity could have even been overestimated in our mouse experimental protocol. Indeed, it has been shown that murine cells have a lower permissivity to lentiviral vector transduction than other mammal cells, including human cells (data not shown and [4, 45]). Avian cells show a better permissivity to transduction than murine cells (data not shown) allowing to predict that minute lentiviral vector vaccine doses would be effective in fowl. Furthermore, the 50% protective dose of 6.2 ng of p24 antigen of TRIP/sE_{WNV} vector particles was calculated under the most stringent challenge conditions (early challenge at day 7 post-vaccination and a lethal dose of 1,000 LD_{50}). Given that total antibody concentrations increase by ten-fold between day 7 and 14, the 50% protective dose would probably have been even lower than 6.2 ng if calculated only one week later. Thirdly, by virtue of the ubiquitous tropism of the VSV-G envelope used for pseudotyping the vector particles [37], the lentiviral vector vaccine can theoretically be used, with no modification, in any vertebrate species: horses, fowl and zoo mammals at risk. A final consideration is that the protective immunity conferred by the vector is sterilizing, no viral replication takes place after WNV challenge. This could represent an important advantage if the vaccine were to be used for bird-immunization. Indeed, while horses, humans and other mammals are believed to be dead-end hosts of WNV infection, birds are known to be amplifying hosts and participate in the maintenance of an epidemic [30]. Moreover, recent literature shows that experimentally WNV infected hamsters that survive the acute illness can continue shedding infectious WNV in urine for up to 52 days [46]. If this can be generalized to other WNV susceptible mammals, a sterilizing vaccine could become crucial for the control of the WNV epidemic.

Several WNV vaccines for veterinary use have been proposed. One licensed in the US in 2003 for horses is an inactivated and adjuvanted virus (Fort Dodge website: www.equinewestnile.com). This inactivated preparation elicits low magnitude humoral responses in horses and thus requires several injections, followed by annual boosts. This killed virus vaccine does not elicit neutralizing antibodies in Chilean Flamingos and Red-tail hawks [47]. A recombinant canary pox encoding the preM/E proteins of the WNV has also been licensed in 2004 [48]. However this candidate also requires two injections at 5 weeks intervals. Neither vaccine provides absolute protection to the vaccinated horses. Other strategies, such as naked DNA encoding pre M and E proteins of WNV [28] are also envisioned.

Candidate vaccines against WNV are for the most part being developed for human use. A live measles vaccine expressing the secreted form of the E-glycoprotein of WNV has been shown to efficiently protect mice against WNV encephalitis [29]. Two recombinant live-attenuated vaccines, chimeric WNV/yellow fever and WNV/Dengue 4, containing the pre M and E genes of WNV cloned into the backbone of either the 17D vaccine strain of yellow fever virus or the dengue 4 virus, have been tested. Preclinical studies in mice and macaques show that both vaccines are protective against WNV challenge [49-51]. However, the use of chimeric live-attenuated virus might pose safety concerns: recombination between different flavivirus species is possible, as demonstrated by naturally occurring recombinants flaviviruses [52, 53].

The potential use of lentiviral vector-based vaccines, for human or veterinary use, will require careful design of the vector and rigorous pre-clinical safety studies. Safety concerns about the use of integrative vectors are justified in front of the recent reports of leukemia cases in the SCID. X1 trial. These severe adverse effects are directly linked to the integration of the Moloney-derived retroviral vector at close proximity of the LMO2 proto-oncogene in hematopoietic stem cells [54].

However, vaccination applications of lentiviral vectors present

lower risks. As opposed to stem cell-based gene therapy, which involves extensive proliferation of transduced cells and persistence of the genetic modification for the entire life of the patient, transduced cells in a vaccination scenario express the relevant antigen, and thus are targets of the elicited immune response. Cells expressing the antigen, whether APCs or not, are thought to be eliminated from the vaccinated organism within weeks or months. Furthermore, in the particular case of vaccination by sub-cutaneous or intravenous injections, it has been shown that VSV-G pseudotyped lentiviral vector particles target essentially DCs [13, 18], a non dividing professional APC with short *in vivo* half life upon activation. Nevertheless, both vector cell tropism and lack of persistence of vaccination vector sequences, depending on the route of injection and vector dose, will have to be carefully addressed for each vaccination protocol.

Lentiviral vectors appear to be promising tools for vaccination against West Nile Virus and likely other zoonoses or emerging pathogens.

Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

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WHAT IS CLAIMED IS:

1. Use of a lentiviral vector comprising a heterologous nucleic acid encoding an antigen, and wherein expression of the antigen in a cell of an animal elicits a humoral response in said animal, for the preparation of a medicament able to produce antibodies when administered to said animal.

2. The use of claim 1, wherein the antigen is selected in the group consisting of a surface antigen and a membrane antigen.

3. The use of claim 1 or claim 2, wherein the humoral response induces a protective immunity in an animal in need thereof, said protective immunity being either a long-lasting protective immunity or a sterilizing immunity.

4. The use of any of claims 1 to 3, wherein the expression of the antigen induces protective immunity against a pathogenic microorganism.

5. The use of claim 4, wherein the pathogenic microorganism is a flavivirus.

6. The use of claim 5, wherein the pathogenic microorganism is West Nile Virus.

7. The use of any of claims 1 to 6, wherein the heterologous nucleic acid encodes an envelope E-glycoprotein from West Nile Virus, a fragment thereof, or a variant of said envelope E-glycoprotein from West Nile Virus.

8. The use of claim 7, wherein said fragment is selected in the group consisting of carboxyl terminal-truncated E glycoprotein from West Nile Virus lacking the transmembrane anchoring region and carboxyl terminal-truncated E glycoprotein comprising residues 1-441 of the envelope E-glycoprotein from West Nile Virus strain IS-98-ST1.

9. Use of a lentiviral vector comprising a heterologous nucleic acid selected in the group consisting of an heterologous nucleic acid encoding a surface protein from a microorganism, or a fragment thereof, and a variant of a surface membrane protein or a fragment thereof, with an acceptable

physiological carrier and/or an adjuvant, for the preparation of a vaccine against a microorganism infection, able to be administered to an animal in need thereof, one or more times.

10. The use of claim 9, wherein the microorganism is West Nile Virus, and wherein the heterologous nucleic acid encodes envelope E-glycoprotein from West Nile Virus, a variant thereof or a fragment thereof, with an acceptable physiological carrier and/or an adjuvant.

11. The use of claim 9 or claim 10, wherein the lentiviral vector is administered by an intraperitoneal route, intravenous route, intramuscular route, oral route, mucosal route, sublingual route, subcutaneous route, intranasal route or intradermic route.

12. The use of claim 11, wherein the lentiviral vector is administered by an intramuscular route, and wherein the animal in need thereof is a horse.

13. The use of claim 11, wherein the lentiviral vector is administered by an intranasal route or an intradermic route, and wherein the animal in need thereof is a human.

14. The use of any of claims 9 to 13, wherein the heterologous nucleic acid encodes a fragment of an envelope E-glycoprotein from West Nile Virus selected in the group consisting of: a carboxyl terminal-truncated E-glycoprotein from West Nile Virus lacking the transmembrane anchoring region and a carboxyl terminal-truncated E glycoprotein comprising residues 1-441 of the envelope E-glycoprotein from West Nile Virus strain IS-98-ST1.

15. The use of any of claims 9 to 14, wherein the lentiviral vector is administered one time and comprises a dose of vector particles equivalent to 0.5 ng to 5000 ng of p24 antigen, preferably a dose of vector particles equivalent to 0.5 ng to 50 ng of p24 antigen, and more preferably a dose of vector particles equivalent to 50 to 500 ng of p24 antigen.

16. An immunogenic composition comprising a lentiviral vector, wherein the lentiviral vector comprises a heterologous nucleic acid encoding an antigen in an amount sufficient to induce an immunogenic or protective response *in vivo*,

and a pharmaceutically acceptable carrier therefor.

17. The immunogenic composition of claim 16, wherein the heterologous nucleic acid encodes a surface protein from a microorganism, or a fragment thereof.

18. The immunogenic composition of claim 16, wherein the heterologous nucleic acid encodes envelope E-glycoprotein from a flavivirus, or a fragment thereof.

19. The immunogenic composition of claim 18, wherein the heterologous nucleic acid encodes envelope E-glycoprotein from a West Nile virus or a fragment thereof.

20. The immunogenic composition of claim 19, wherein the heterologous nucleic acid is selected in the group consisting of a heterologous nucleic acid encoding carboxyl terminal-truncated E glycoprotein from West Nile Virus lacking the transmembrane anchoring region, heterologous nucleic acid encoding a carboxyl terminal-truncated E glycoprotein comprising residues 1-441 of the envelope E-glycoprotein from West Nile Virus strain IS-98-ST1, and heterologous nucleic acid encoding a variant of envelope E-glycoprotein from West Nile Virus.

21. A lentiviral vector that directs the expression of a heterologous nucleic acid, wherein the heterologous nucleic acid encodes envelope E-glycoprotein from West Nile Virus, a variant thereof or fragments thereof, and wherein the vector comprises a promoter selected from cytomegalovirus immediate early promoter, CAG promoter, EF1alpha promoter, PGK promoter, SVero promoter, and MND promoter.

22. The lentiviral vector of claim 21, wherein the heterologous nucleic acid is selected in the group consisting of a heterogenous nucleic acid encoding carboxyl terminal-truncated E glycoprotein from West Nile Virus lacking the transmembrane anchoring region and heterogenous nucleic acid encoding carboxyl terminal-truncated E glycoprotein comprises residues 1-441 of the envelope E-glycoprotein from West Nile Virus strain IS-98-ST1.

23. The lentiviral vector of claim 22, wherein the vector comprises

pTRIP/sE$_{WNV}$.

24. A substantially purified host cell transfected or transduced with the lentiviral vector of any of claims 21 to 23.

25. A method for the production of envelope E-glycoprotein polypeptide from West Nile Virus, a variant thereof or a fragment thereof, comprising culturing a host cell of claim 24 under conditions promoting expression, and recovering the polypeptide from the host cell or the culture medium.

26. A lentiviral vector that directs the expression of a heterologous nucleic acid, wherein the vector comprises cytomegalovirus immediate early promoter, and wherein the heterologous nucleic acid comprises green fluorescent protein.

27. A substantially purified host cell transfected or transduced with the lentiviral vector of claim 26.

28. Use of a lentiviral vector comprising a heterologous nucleic acid encoding an antigen, and wherein the antigen elicits antibodies against the pathogenic agent, for the preparation of a vaccine against a pathogenic agent, able to be administered to an animal in need thereof.

29. The use of claim 28, wherein administration of the lentiviral vector elicits long-lasting humoral immunity or neutralizing antibodies against the pathogenic agent.

30. The use of any of claims 28 and 29, wherein the lentiviral vector comprises a nucleic acid encoding envelope E-glycoprotein from West Nile Virus, or a fragment thereof, and wherein the pathogenic agent is West Nile Virus.

31. The use of claim 30, wherein envelope E-glycoprotein from West Nile Virus lacks a transmembrane anchoring region.

32. The use of any of claims 28 to 31, wherein the administration of lentiviral vector comprises at least one dose of vector particles equivalent to 0.5 ng to 5000 ng of p24 antigen.

33. The use of claim 3, claim 9 or claim 28, wherein the animal in need thereof is selected from humans, horses, birds, poultry, pigs, cattle, rodents, pets, and reptiles.

34. The use of claim 7, claim 9, claim 20 or claim 21, or the method of claim 25, wherein the heterologous nucleic acid encodes a variant of envelope E-glycoprotein from West Nile Virus and hybridizes under conditions of stringent hybridization to either strand of a nucleic acid from West Nile Virus strain IS-98-ST1 encoding envelope E-glycoprotein.