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(54) DENGUE AND WEST NILE VIRUS PROTEINS AND GENES AND THEIR THERAPEUTIC APPLICATION

PROTEINE UND GENE VOM NEW DENGUE UND WEST NILE VIRUS UND IHRE THERAPEUTISCHE VERWENDUNG

PROTEINES DES VIRUS DU WEST NILE ET DE LA DENGUE, GENES CODANT CES PROTEINES ET LEUR UTILISATION THERAPEUTIQUE

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Description

FIELD OF THE INVENTION

[0001] The application discloses West-Nile virus (WNV) and/or Dengue virus derived peptides, and more particularly polypeptides or polynucleotides derived from WNV and/or Dengue virus polypeptides or polynucleotides and their use in the preparation of compositions and vaccines. The present invention is concerned with compositions, vaccines and methods for providing an immune response and/or a protective immunity to animals against a West-Nile virus or a Dengue virus.

BACKGROUND OF THE INVENTION

[0002] Flaviviridae are arboviruses (arthropod-borne virus) mainly transported by mosquitoes and blood-sucking ticks. They are small encapsidated viruses and their genomes consist of Infectious single-stranded and linear RNA of positive polarity. In Man, flaviviruses cause deadly hemorrhagic fever or meningoencephalitis. Yellow fever, dengue fever and Japanese encephalitis are the main tropical flaviviroses. Other important human flaviviroses are Saint Louis encephalitis, tick-born European encephalitis and West Nile fever.

[0003] West Nile fever is a zoonosis associated with a flavivirus which was first isolated in Uganda In 1937. Its transmission cycle calls for birds as the main reservoir and for blood sucking mosquitoes of the Culex genus as vectors. Migratory viremic birds transport the virus to far-away regions where they transmit it anew to ornithophile mosquitoes of the Culex genus. Many species of mammals are permissive for the West Nile virus. Horses are particularly sensitive to the disease but do not participate in the cycle of transmission. West Nile fever is endemic in Africa, Asia, Europe and Australia. Phylogenic studies have revealed the existence of two strains of viruses : viral line 1 has a worldwide distribution, and viral line 2 is essentially African. Viral line 1 was responsible for enzooties in Romania (1996), Russia (1999), Israel (1998-2000) and more recently in North America where the virus had never been detected before 1999. The viral strains isolated during the recent epidemics in Israel and the United-States are more than 99,7 % identical. In the Middle-East and North America, where the virus has taken root, an important bird mortality rate has been observed among infected birds, notably in Corvidae. In North America, over 4000 subjects were infected with the West Nile virus, 250 of which died between the months of August and December 2002. At the present time, zoonosis is observed in all regions of the United States. At the moment, there exists no human vaccine or specific therapy against West Nile fever.

[0004] In temperate and sub-tropical regions, human infections may occur during the fall season. When a subject is bitten by an infected mosquito, the incubation period lasts approximately one week but less than 20 % of people infected with the West Nile virus ever go on to clinical manifestations. In its benignant form, the viral infection manifests itself by an undifferentiated febrile state associated with muscular weakness, headaches and abdominal pain. In less than 1% of infected subjects, encephalitis or acute aseptic meningitis may occur. Splenomegaly, hepatitis, pancreatitis and myocarditis are also observed. Flask paralyses similar to a poliomyelitic syndrome have recently been reported, but fatal cases of viral encephalitis (5% of patients having severe neurological disorders) mainly concern fragile subjects and the aged. Inter-human transmission of the virus has also recently been observed in the United-States in subjects having undergone organ transplants or having been perfused with contaminated blood products. Intrauterine transmission of the virus has been reported in the United-States. The development of a human vaccine against the West Nile fever is a priority in view of the fact that the zoonosis has taken root in North America and is expected to propagate in the coming months to Central America, South America and the Caribbean where dengue fever and yellow fever are already rampant.

[0005] Therefore, there is a need for West-Nile virus (WNV) and/or Dengue virus derived peptides, and more particularly to polypeptides or polynucleotides derived from WNV and/or Dengue virus polypeptides or polynucleotides and their use in the preparation of compositions and vaccines..

[0006] The present invention fulfils these needs and also other needs which will be apparent to those skilled in the art upon reading the following specification.

[0007] Davis B. et al (Journal of Virology, May 2001, p4040-4047) discloses a recombinant expression plasmid expressing the pre-M and E proteins of a West Nile Virus strain. According to this publication, a single intramuscular injection of the vector induced protective immunity in mice and horses.

[0008] International patent application WO 90/01946 describes Vaccinia viruses expressing flavivirus antigenic proteins, in particular antigenic proteins from a Dengue type 4 virus, a Japanese B or a tick-borne encephalitis virus. In particular it discloses Vaccinia viruses containing a coding sequence for the capsid membrane and envelope antigens of the Dengue virus, that expressed the corresponding antigens and enabled protection of immunized mice..

[0009] Wang Z. et al (Vaccine 19 (2001)) describes recombinant measles viruses expressing either the HN or the F surface glycoproteins of Mumps virus or the ENV, GAG or POL proteins of the Simian Immunodeficiency Virus.

[0010] International patent application WO 2004/001051 with a filing date of June 30, 2003, discloses recombinant measles virus expressing the envelope or the pre-M antigen of the West Nile Virus. It discloses the secreted envelope

polypeptide of the West Nile Virus but the disclosure in this respect does not benefit from the priority date of said international patent application.

[0011] Fonseca B. et al (Vaccine 1994 Vol 12 Number 3) describes recombinant Vaccinia viruses co-expressing Dengue 1 glycoproteins pre-M and E. Tthe publication states that the thus obtained viruses elicited the production of neutralizing antibodies in mice inoculated with the recombinant viruses.

SUMMARY OF THE INVENTION

[0012] The application discloses West-Nile virus and/or Dengue virus derived polypeptides, and a purified polypeptide wherein it derives from a West-Nile virus antigen or a Dengue virus antigen.

[0013] The application discloses a purified polyclonal or monoclonal antibody, capable of specifically binding to a polypeptide disclosed.

[0014] The application discloses a purified polynucleotide sequence coding for the polypeptide disclosed and its use for detecting the presence or absence of a West-Nile virus antigen or a Dengue virus antigen in a biological sample.

[0015] The invention concerns a recombinant viral vector which is a recombinant measles virus comprising a polynucleotide sequence disclosed.

[0016] An object of the invention is a recombinant measles virus capable of expressing a polypeptide of the invention or comprising, in its genome, a polynucleotide disclosed.

[0017] Yet, another object of the invention relates to a pharmaceutical composition comprising:

a) at least one component selected from the group consisting of:

- a recombinant measles virus of the invention; and

b) a pharmaceutically acceptable vehicle or carrier.

[0018] Another object of the invention concerns the use of the pharmaceutical composition of the invention, as an anti-West-Nile virus and/or an anti-Dengue virus agent, or for the preparation of an anti-West-Nile virus and/or an anti-Dengue virus vaccine.

[0019] Another object of the invention relates to a host cell comprising a recombinant viral vector as defined above.

[0020] Furthermore, another object of the invention concerns a method of producing a recombinant virus for the preparation of an anti-West-Nile virus vaccine or an anti-Dengue virus vaccine, the method comprising the steps of:

a) providing a host cell as defined above;
b) placing the host cell from step a) in conditions permitting the replication of a recombinant virus capable of expressing a polypeptide disclosed ; and
c) isolating the recombinant virus produced in step b).

[0021] The application discloses a West-Nile virus neutralization assay, comprising the steps of:

a) contacting VERO cells with West-Nile virus and an antibody;
b) culturing said VERO cells under conditions which allow for West-Nile virus replication; and
c) measuring reduction of West-Nile virus replication foci on said VERO cells.

[0022] The application discloses a method for treating and/or preventing a WNV- or Dengue virus-infection in an animal, the method comprising the step of administering to the animal an effective amount of at least one element selected from the group consisting of :

- a polypeptide or a functional derivative thereof as defined above;
- an antibody as defined above;
- an expression vector as defined above;
- a polynucleotide or a fragment thereof as defined above;
- a recombinant viral vector as defined above; and
- a recombinant measles virus as defined above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023]

Figure 1 shows the nucleic acid sequence encoding the secreted glycoprotein E from WNV and identified as SEQ ID NO. 1.

Figure 2. shows the amino acid sequence of the secreted glycoprotein E from WNV and identified as SEQ ID NO 5.

Figure 3 shows the nucleic acid sequence encoding the preM plus E glycoproteins from WNV and identified as SEQ ID NO. 2.

Figure 4 shows the amino acid sequence of the preM plus E glycoproteins from WNV and identified as SEQ ID NO 6.

Figure 5 shows the nucleic acid sequence encoding the preM-E gene from Dengue type 1 virus and identified as SEQ ID NO. 4.

Figure 6 shows the amino acid sequence of the preM-E gene from Dengue type 1 virus and identified as SEQ ID NO 8.

Figure 7 is a schematic map of the pTM-MVSchw recombinant plasmids according to preferred embodiments of the invention.

Figure 8 shows the expression of sEWNV by MVSchw-sE_{WNV} recombinant MV and virus growth in Vero cells. (**A**) Schematic diagram of MVschw-sE_{WNV} and virus growth. The IS-98-ST1 cDNA coding for sE_{WNV} was inserted into the Schwarz MV genome between the *BsiW1* and *BssHII* sites of the ATU at position 2. The MV genes are indicated: N (nucleoprotein), PVC (phosphoprotein and V, C proteins), M (matrix), F (fusion), H (hemagglutinin), L (polymerase). T7: T7 RNA polymerase promoter; hh : hammerhead ribozyme, T7t: T7 RNA polymerase terminator; δ: hepatitis delta virus (HDV) ribozyme; ATU : additional transcription unit. (**B**) Growth curves of MV. Vero cells were infected with MVschw (open box) or MVschw-sE_{WNV} (black box) at a multiplicity of infection (m.o.i) of 0.01 $TCID_{50}$/cell. At various times post-infection, infectious virus particles were titered as described in the Methods. (**C**) Immunofluorescence staining of sEWNV glycoprotein in syncitia of MVschw-sE_{WNV}-infected Vero cells fixed 36 h post-infection. Cells were permeabilized (A, B) or not (C, D) with Triton X-100 and then immunostained using anti-WNV HMAF. Magnification: x 1000. No positive signal was observed in MVschw-infected cells. (**D**) Radioimmunoprecipitation (RIP) assay showing the release of sE_{WNV} from MVschw-sE_{WNV}-infected cells. Vero cells were infected with WNV strain IS-98-ST1 (m.o.i of 5) for 24 h, MVschw (m.o.i. of 0.1), MVschw-sE_{WNV} (m.o.i. of 0.1) for 40 h, or mock-infected (MI). Radiolabeled supernatants and cell lysates were immunoprecipitated with specific anti-MV (α-MV) or anti-WNV (α-WNV) polyclonal antibodies. WNV E glycoprotein (open arrow head) and sE_{WNV} (black arrow head) are shown.

Figure 9 shows anti-MVSchw-sE_{WNV} antibodies recognizing the WNV E glycoprotein. Vero cells were infected with WNV strain IS-98-ST1 (WNV) or mock-Infected (No virus). Labeled cell lysates were immunoprecipitated with pooled immune sera (dilution 1:100) from mice inoculated with WNV, MVSchw, MVSchw-sE_{WNV} as described in the legend to Fig. 8D. Specific anti-lymphochoriomeningitis virus (LCMV) antibodies were used as a negative control. WNV structural glycoproteins prM and E and non structural proteins NS3, NS5, NS2A and NS2B are shown. p.c., post-challenge.

[0024] SEQ ID No. 3, 7, 13 and 14 which were originally disclosed with respect to MS1 protein from WNV are not part of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0025] The application discloses West-Nile virus (WNV) and/or Dengue virus derived peptides, and more particularly polypeptides or polynucleotides derived from WNV and/or Dengue virus polypeptides or polynucleotides and their use in the preparation of compositions and vaccines. The present invention is concerned with compositions, vaccines and methods for providing an immune response and/or a protective immunity to animals against a West-Nile virus or a Dengue virus.

[0026] As used herein, the term "immune response" refers to the T cell response or the increased serum levels of antibodies to an antigen, or presence of neutralizing antibodies to an antigen, such as a WNV or a Dengue virus antigen. The term "immune response" is to be understood as including a humoral response and/or a cellular response and/or an inflammatory response.

[0027] An "antigen" refers to a molecule, such as a protein or a polypeptide, containing one or more epitopes that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term is also used interchangeably with "immunogen".

[0028] The term "protection" or "protective immunity" refers herein to the ability of the serum antibodies and cellular response induced during immunization to protect (partially or totally) against against a West-Nile virus or a Dengue virus. Thus, an animal immunized by the compositions or vaccines of the invention will experience limited growth and spread of an infectious WNV or Dengue virus.

[0029] As used herein, the term "animal" refers to any animal that is susceptible to be infected by a West-Nile virus or a Dengue virus. Among the animals which are known to be potentially infected by these viruses, there are, but not limited to, humans, birds and horses.

1. Polynucleotides and polypeptides

[0030] In a first embodiment, the application discloses a purified polypeptide characterized in that it derives from a West-Nile virus antigen or a Dengue virus antigen or functional derivative thereof. As can be appreciated, a protein/peptide is said to "derive" from a protein/peptide or from a fragment thereof when such protein/peptide comprises at least one portion, substantially similar in its sequence, to the native protein/peptide or to a fragment thereof.

[0031] The West-Nile virus antigen used in the recombinant virus of the present invention is preferably selected from the group consisting of secreted envelope glycoprotein (E), heterodimer glycoproteins (PreM-E). More specifically, the secreted envelope glycoprotein (E) comprises the sequence of SEQ ID NO: 5 the heterodimer glycoproteins (PreM-E) comprises the sequence of SEQ ID NO: 6.

[0032] The Dengue virus antigen used in the recombinant virus of the invention is preferably selected from the group consisting of secreted envelope glycoprotein (E), heterodimer glycoproteins (PreM-E). More specifically, the heterodimer glycoproteins (PreM-E) comprises the sequence of SEQ ID NO: 8.

[0033] According to a preferred embodiment, the polypeptide used in the recombinant virus of the present invention has an amino acid sequence having at least 80% homology, or even preferably 85% homology to part or all of SEQ ID NO:5 of SEQ ID NO:6 or of SEQ ID NO:8.

[0034] A functional derivative", as is generally understood and used herein, refers to a protein/peptide sequence that possesses a functional biological activity that is substantially similar to the biological activity of the whole protein/peptide sequence. In other words, it refers to a polypeptide or fragment(s) thereof that substantially retain the same biological function as the polypeptides of SEQ ID Nos: 5, 6, 8. A functional derivative of a protein/peptide may or may not contain post-translational modifications such as covalently linked carbohydrate, if such modification is not necessary for the performance of a specific function. The term "functional derivative" is intended to the "fragments", "segments", "variants", "analogs" or "chemical derivatives" of a protein/peptide. As used herein, a protein/peptide is said to be a "chemical derivative" of another protein/peptide when it contains additional chemical moieties not normally part of the protein/peptide, said moieties being added by using techniques well known in the art. Such moieties may improve the protein/peptide solubility, absorption, bioavailability, biological half life, and the like. Any undesirable toxicity and side-effects of the protein/peptide may be attenuated and even eliminated by using such moieties.

[0035] Yet, more preferably, the polypeptide comprises an amino acid sequence substantially, the same or having 100% identity with SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:8.

[0036] One can use a program such as the CLUSTAL program to compare amino acid sequences. This program compares amino acid sequences and finds the optimal alignment by inserting spaces in either sequence as appropriate. It is possible to calculate amino acid identity or homology for an optimal alignment. A program like BLASTx will align the longest stretch of similar sequences and assign a value to the fit It is thus possible to obtain a comparison where several regions of similarity are found, each having a different score. Both types of identity analysis are contemplated in the present invention.

[0037] As used herein, the term "polypeptide(s)" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide(s)" refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers and to longer chains generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptide(s)" include those modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation, selenoylation, sulfation and transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance: PROTEINS-STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W.H. Freeman and Company, New York (1993); Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 In POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., Meth. Enzymol, 182:628-848 (1990); and Rattan et al., Protein Synthesis: Posttranslational Modifications and Aging, Ann. N.Y. Acad. Sci. 663: 48-62(1992). Polypeptides may be branched or cyclic, with or without branching. Cyclic, branched and branched circular polypeptides may result from post-translational natural processes and may be

made by entirely synthetic methods, as well.

[0038] With respect to protein or polypeptide, the term "isolated polypeptide" or "isolated and purified polypeptide" is sometimes used herein. This term refers primarily to a protein produced by expression of an isolated polynucleotide molecule contemplated by invention. Alternatively, this term may refer to a protein which has been sufficiently separated from other proteins with which it would naturally be associated, so as to exist in "substantially pure" form.

[0039] The term "substantially pure" refers to a preparation comprising at least 50-60% by weight the compound of interest (e. g., nucleic acid, oligonucleotide, protein, etc.). More preferably, the preparation comprises at least 75% by weight, and most preferably 90-99% by weight, the compound of interest.

[0040] Purity is measured by methods appropriate for the compound of interest (e.g. chromatographic methods, agarose or polyacrylamide gel electrophoresis, HPLC analysis, and the like).

[0041] The application discloses a purified polynucleotide encoding a polypeptide disclosed Therefore, the polynucleotide has a nucleic acid sequence which is at least 65% identical, more particularity 80% identical and even more particularly 95% identical to part or all of any one of SEQ ID NO 1, 2 to 4 or functional fragments thereof.

[0042] A "functional fragment", as is generally understood and used herein, refers to a nucleic acid sequence that encodes for a functional biological activity that is substantially similar to the biological activity of the whole nucleic acid sequence. In other words, it refers to a nucleic acid or fragment(s) thereof that substantially retains the capacity of encoding for a polypeptide disclosed.

[0043] The term "fragment" as used herein refer to a polynucleotide sequence (e.g., cDNA) which is an isolated portion of the subject nucleic acid constructed artificially (e.g., by chemical synthesis) or by cleaving a natural product into multiple pieces, using restriction endonucleases or mechanical shearing, or a portion of a nucleic acid synthesized by PCR, DNA polymerase or any other polymerizing technique well known in the art, or expressed in a host cell by recombinant nucleic acid technology well known to one of skill in the art.

[0044] With reference to potynudeotides disclosed in the application the term "isolated polynucleotide" is sometimes used. This term, when applied to DNA, refers to a DNA molecule that is separated from sequences with which it is immediately contiguous (in the 5' and 3' directions) in the naturally occurring genome of the organism from which it was derived. For example, the "isolated polynucleotide" may comprise a DNA molecule inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a procaryote or eucaryote. An "isolated polynucleotide molecule" may also comprise a cDNA molecule.

[0045] Amino acid or nucleotide sequence "identity" and "similarity" are determined from an optimal global alignment between the two sequences being compared. An optimal global alignment is achieved using, for example, the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol. Biol. 48:443-453). "Identity" means that an amino acid or nucleotide at a particular position in a first polypeptide or polynucleotide is identical to a corresponding amino acid or nucleotide in a second polypeptide or polynucleotide that is in an optimal global alignment with the first polypeptide or polynucleotide. In contrast to identity, "similarity" encompasses amino acids that are conservative substitutions. A "conservative" substitution is any substitution that has a positive score in the blosum62 substitution matrix (Hentikoff and Hentikoff, 1992, Proc. Natl. Acad. Sci. USA 89: 10915-10919). By the statement "sequence A is n% similar to sequence B" is meant that n% of the positions of an optimal global alignment between sequences A and B consists of identical residues or nucleotides and conservative substitutions. By the statement "sequence A is n% identical to sequence B" is meant that n% of the positions of an optimal global alignment between sequences A and B consists of identical residues or nucleotides.

[0046] As used herein, the term "polynucleotide(s)" generally refers to any polyribonucleotide or poly-deoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. This definition includes, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, cDNA, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or tripie-stranded regions, or a mixture of single- and double-stranded regions. In addition, "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term "polynucleotide(s)" also includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotide(s)" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art "Polynucleotide(s)" embraces short polynucleotides or fragments comprising at least 6 nucleotides often referred to as oligonucleotide(s). The term "polynucleotide(s)" as it is employed herein thus embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including, for example, simple and complex cells

which exhibits the same biological function as the polypeptide encoded by any one of SEQ ID NOS.1, 2, 4. The term "polynucleotide(s)" also embraces short nucleotides or fragments, often referred to as "oligonucleotides", that due to mutagenesis are not 100% identical but nevertheless code for the same amino acid sequence.

2. Vectors and Cells

[0047] In a third embodiment, the invention is also directed to a host, such as a genetically modified cell, comprising the recombinant virus according to the invention and more preferably, a host capable of expressing the polypeptide encoded by the polynucleotide comprised in said virus. Even more preferably, the present invention is concerned with a host cell that comprises a recombinant viral vector as defined herein below.

[0048] The host cell may be any type of cell (a transiently-transfected mammalian cell line, an isolated primary cell, or insect cell, yeast (*Saccharomyces cerevisiae*, Ktuyveromyces lactis, *Pichia pastoris*), plant cell, microorganism, or a bacterium (such as *E. coli*). The following biological deposit relating to MEF/3T3.Tet-Off/prME.WN# h2 cell line comprising an expression vector encoding for pseudo-particles of WNV strain IS-98-ST1 composed of prME complexed glycoproteins was registered at the Collection Nationale des Cultures de Microorganismes (CNCM) under accession numbers I-3018 on May 2, 2003.

[0049] The application discloses cloning or expression vector comprising a polynucleotide sequence as defined above.

[0050] As used herein, the term "vector" refers to a polynucleotide construct designed for transduction/transfection of one or more cell types. Vectors may be, for example, "cloning vectors" which are designed for isolation, propagation and replication of inserted nucleotides, "expression vectors" which are designed for expression of a nucleotide sequence in a host cell, or a "viral vector" which is designed to result in the production of a recombinant virus or virus-like particle, or "shuttle vectors", which comprise the attributes of more than one type of vector.

[0051] A number of vectors suitable for stable transfection of cells and bacteria are available to the public (e.g. plasmids, adenoviruses, baculoviruses, yeast baculoviruses, plant viruses, adeno-associated viruses, retroviruses, Herpes Simplex Viruses, Alphaviruses, Lentiviruses), as are methods for constructing such cell lines. It will be understood that the application discloses any type of vector comprising any of the polynucleotide molecule disclosed.

[0052] According to the invention the vector is a recombinant viral vector which is a recombinant virus comprising a polynucleotide sequence as defined above. The recombinant virus is a live attenuated virus or a defective measles virus. More preferably, the recombinant measles virus is for instance the Schwarz measles virus strain, which is capable of expressing a polypeptide as defined above or comprises, in its genome, a polynucleotide as defined above.

3. Antibodies

[0053] The application discloses purified antibodies that specifically bind to the isolated or purified polypeptide as defined above or fragments thereof. The antibodies may be prepared by a variety of methods using the polypeptides described above. For example, the West-Nile or Dengue virus antigen, or antigenic fragments thereof, may be administered to an animal in order to induce the production of polyclonal antibodies. Alternatively, antibodies used as described herein may be monoclonal antibodies, which are prepared using hybridoma technology (see, e.g., Hammerling et al., In Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, NY, 1981).

[0054] As mentioned above, the application discloses antibodies that specifically bind to a West-Nile antigen or a Dengue virus antigen, or fragments thereof. In particular, the application discloses "neutralizing" antibodies. By "neutralizing" antibodies is meant antibodies that interfere with any of the biological activities of any of the WNV antigen or Dengue virus antigen. Any standard assay known to one skilled in the art may be used to assess potentially neutralizing antibodies. Once produced, monoclonal and polyclonal antibodies are preferably tested for specific WNV or Dengue virus proteins recognition by Western blot, immunoprecipitation analysis or any other suitable method.

[0055] Antibodies that recognize WNV or Dengue virus proteins expressing cells and antibodies that specifically recognize WNV or Dengue virus proteins (or functional fragments thereof), such as those described herein, are considered useful to the invention. Such an antibody may be used in any standard immunodetection method for the detection, quantification, and purification of WNV or Dengue virus proteins. The antibody may be a monoclonal or a polyclonal antibody and may be modified for diagnostic purposes. The antibodies may, for example, be used in an immunoassay to monitor WNV or Dengue virus proteins expression levels, to determine the amount of WNV or Dengue virus proteins or fragment thereof in a biological sample and evaluate the presence or not of a WNV or Dengue virus. In addition, the antibodies may be coupled to compounds for diagnostic and/or therapeutic uses such as gold particles, alkaline phosphatase, peroxidase for imaging and therapy. The antibodies may also be labeled (e.g. immunofluorescence) for easier detection.

[0056] With respect to antibodies disclosed in the application the term "specifically binds to" refers to antibodies that bind with a relatively high affinity to one or more epitopes of a protein of interest, but which do not substantially recognize and bind molecules other than the one(s) of interest. As used herein, the term "relatively high affinity" means a binding

affinity between the antibody and the protein of interest of at least 10^6 M^{-1}, and preferably of at least about 10^7 M^{-1} and even more preferably 10^8 M^{-1} to 10^{10} M^{-1}. Determination of such affinity is preferably conducted under standard competitive binding immunoassay conditions which is common knowledge to one skilled in the art. As used herein, "antibody" and "antibodies" include all of the possibilities mentioned hereinafter: antibodies or fragments thereof obtained by purification, proteolytic treatment or by genetic engineering, artificial constructs comprising antibodies or fragments thereof and artificial constructs designed to mimic the binding of antibodies or fragments thereof. Such antibodies are discussed in Colcher et al. (Q J Nucl Med 1998; 42: 225-241). They include complete antibodies, $F(ab')_2$ fragments, Fab fragments, Fv fragments, scFv fragments, other fragments, CDR peptides and mimetics. These can easily be obtained and prepared by those skilled in the art. For example, enzyme digestion can be used to obtain $F(ab')_2$ and Fab fragments by subjecting an IgG molecule to pepsin or papain cleavage respectively. Recombinant antibodies are also disclosed.

[0057] Alternatively, the antibody disclosed may be an antibody derivative. Such an antibody may comprise an antigen-binding region linked or not to a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain or heavy chain variable domain. Typically, the antibody comprises both light and heavy chain variable domains, that can be inserted in constructs such as single chain Fv (scFv) fragments, disulfide-stabilized Fv (dsFv) fragments, multimeric scFv fragments, diabodies, minibodies or other related forms (Colcher et al. Q J Nucl Med 1998; 42: 225-241). Such a derivatized antibody may sometimes be preferable since it is devoid of the Fc portion of the natural antibody that can bind to several effectors of the immune system and elicit an immune response when administered to a human or an animal. Indeed, derivatized antibody normally do not lead to immuno-complex disease and complement activation (type III hypersensitivity reaction).

[0058] Alternatively, a non-immunogiobulin region is fused to the antigen-binding region of the antibody disclosed. The non-immunoglobulin region is typically a non-immunoglobulin moiety and may be an enzyme, a region derived from a protein having known binding specificity, a region derived from a protein toxin or indeed from any protein expressed by a gene, or a chemical entity showing inhibitory or blocking activity(ies) against WNV or Dengue virus proteins. The two regions of that modified antibody may be connected via a cleavable or a permanent linker sequence.

[0059] Preferably, the antibody diclosed is a human or animal immunoglobulin such as IgG1, IgG2, IgG3, IgG4, IgM, IgA, IgE or IgD carrying rat or mouse variable regions (chimeric), or CDRs (humanized or "animalized"). Furthermore, the antibody disclosed may also be conjugated to any suitable carrier known to one skilled in the art in order to provide, for instance, a specific delivery and prolonged retention of the antibody, either in a targeted local area or for a systemic application.

[0060] The term "humanized antibody" refers to an antibody derived from a non-human antibody, typically murine, that retains or substantially retains the antigen-binding properties of the parent antibody but which is less immunogenic in humans. This may be achieved by various methods including (a) grafting only the non-human CDRs onto human framework and constant regions with or without retention of critical framework residues, or (b) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Such methods are well known to one skilled in the art.

[0061] As mentioned above, the antibody disclosed is immunologically specific to the polypeptide used in the recombinant virus of the present Invention and immunological derivatives thereof. As used herein, the term "Immunological derivative" refers to a polypeptide that possesses an immunological activity that is substantially similar to the immunological activity of the whole polypeptide, and such immunological activity refers to the capacity of stimulating the production of antibodies immunologically specific to the WNV or Dengue virus proteins or derivative thereof. The term "immunological derivative" therefore encompass "fragments", "segments", "variants", or "analogs" of a polypeptide.

4. Compositions and vaccines

[0062] The polypeptides, the polynucleotides coding the same, the recombinant measles virus produced according to the invention, may be used in many ways for the treatment or the prevention of WNV- or Dengue virus-infection.

[0063] The present invention relates to a composition for eliciting an immune response or a protective immunity against a WNV or a Dengue virus. According to a related aspect, the present invention relates to a vaccine for preventing and/or treating a WNV- or Dengue virus-infection. As used herein, the term "treating" refers to a process by which the symptoms of a WNV- or Dengue virus-infection are alleviated or completely eliminated. As used herein, the term "preventing" refers to a process by which a WNV- or Dengue virus-infection is obstructed or delayed. The composition or the vaccine of the invention comprises a recombinant measles virus as defined above and an acceptable carrier.

[0064] As used herein, the expression "an acceptable carrier" means a vehicle for containing the recombinant measles virus of the invention that can be injected into an animal host without adverse effects. Suitable carriers known in the art include, but are not limited to, gold particles, sterile water, saline, glucose, dextrose, or buffered solutions. Carriers may include auxiliary agents including, but not limited to, diluents, stabilizers (i. e., sugars and amino acids), preservatives, wetting agents, emulsifying agents, pH buffering agents, viscosity enhancing additives, colors and the like.

[0065] Further agents can be added to the composition and vaccine of the invention. For instance, the composition

of the invention may also comprise agents such as drugs, immunostimulants (such as α-interferon, β-interferon, γ-interferon, granulocyte macrophage colony stimulator factor (GM-CSF), macrophage colony stimulator factor (M-CSF), interleukin 2 (IL2), interleukin 12 (IL12), and CpG oligonucleotides), antioxidants, surfactants, flavoring agents, volatile oils, buffering agents, dispersants, propellants, and preservatives. For preparing such compositions, methods well known in the art may be used.

[0066] The amount of recombinant measles virus present in the compositions or in the vaccines of the present invention is preferably a therapeutically effective amount. A therapeutically effective amount of the recombinant measles virus of the invention is that amount necessary to allow the same to perform their immunological role without causing, overly negative effects in the host to which the composition is administered. The exact amount of recombinant measles virus to be used and the composition/vaccine to be administered will vary according to factors such as the type of condition being treated, the mode of administration, as well as the other ingredients in the composition.

5. Methods of use

[0067] In a seventh embodiment, the application discloses methods for treating and/or preventing a WNV- or Dengue virus- associated disease or infection in an animal are provided. The method comprises the step of administering to the animal an effective amount of at least one
a recombinant measles virus of the invention.

[0068] The vaccine, and composition of the invention may be given to an animal through various routes of administration. For instance, the composition may be administered in the form of sterile Injectable preparations, such as sterile injectable aqueous or oleaginous suspensions. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparations may also be sterile injectable solutions or suspensions In non-toxic parenterally-acceptable diluents or solvents. They may be given parenterally, for example intravenously, intramuscularly or sub-cutaneously by injection, by infusion or per os. The vaccine and the composition of the invention may also be formulated as creams, ointments, lotions, gels, drops, suppositories, sprays, liquids or powders for topical administration. They may also be administered into the airways of a subject by way of a pressurized aerosol dispenser, a nasal sprayer, a nebulizer, a metered dose inhaler, a dry powder inhaler, or a capsule. Suitable dosages will vary, depending upon factors such as the amount of each of the components in the composition, the desired effect (short or long term), the route of administration, the age and the weight of the animal to be treated. Any other methods well known in the art may be used for administering the vaccine, and the composition of the invention.

[0069] The present invention is also directed to a method of producing a recombinant virus for the preparation of an anti-West-Nile virus vaccine or an anti-Dengue virus vaccine, the method comprising the steps of: .

a) providing a host cell as defined above;
b) placing the host cell from step a) in conditions permitting the replication of a recombinant virus capable of expressing a polypeptide according to the invention; and
c) isolating the recombinant virus produced in step b).

[0070] The application discloses a West-Nile virus neutralisation assay. Accordingly, the assay comprises the steps of:

a) contacting VERO cells with West-Nile virus and an antibody;
b) culturing said VERO cells under conditions which allow for West-Nile virus replication; and
c) measuring reduction of West-Nile virus replication foci on said VERO cells.

EXAMPLES

[0071] The present invention will be more readily understood by referring to the following examples. These examples are illustrative of the wide range of applicability of the present invention and are not intended to limit its scope. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred methods and materials are described.

EXAMPLE 1: CONSTRUCTION OF MEASLES VIRUSES (MV) EXPRESSING WNV AND DEN1 ANTIGENS

[0072] In order to test their capacity as vaccine candidates against WNV infection, recombinant Schwarz measles viruses (MV) expressing these WNV and DEN-1 antigens were constructed: The different genes were introduced in an additional transcription unit in the Schwarz MV cDNA that the inventors previously cloned (pTM-MVSchw) (European Patent Application N° 02291551.6 filed on June 20, 2002). After rescue of the different recombinant Schwarz measles

viruses expressing the WNV and DEN-1 genes, their capacity to protect mice from a lethal WNV intraperitoneal challenge, and monkeys from Dengue virus infection will be tested.

MV vector

[0073] Mass vaccination with live attenuated vaccines has reduced the incidence of measles and its complications dramatically since it was introduced in the 60's. By now, the vaccine has been given to billions of people and is safe and efficacious. It induces a very efficient, life-long CD4, CD8 and humoral immunity after a single injection of 104 TCID50. Moreover, it is easy to produce, cheap, and the means to deliver it worldwide already exist. The safety of this vaccine is due to several factors: i) The stability of the MV genome which explains that reversion to pathogenicity has never been observed. ii) The impossibility for the MV genome to integrate in host chromosomes since viral replication is exclusively cytoplasmic. iii) The production of the vaccine on safe primary chick embryo fibroblastic cells. Thus, live attenuated MV could provide a safe and efficient pediatric vaccination vector.

[0074] MV belongs to the genus Morbillivirus in the family Paramyxoviridae. The Edmonston MV was isolated in 1954 (32), serially passaged on primary human kidney and amnion cells, then adapted to chick embryo fibroblasts (CEF) to produce Edmonston A and B seeds (see (7, 8) for review). Edmonston B was licensed in 1963 as the first MV vaccine. Further passages of Edmonston A and B on CEF produced the more attenuated Schwarz and Moraten viruses (33) whose sequences have recently been shown to be identical (34, 35). Being "reactogenic," Edmonston B vaccine was abandoned in 1975 and replaced by the Schwarz/Moraten vaccine. This is now the most commonly used measles vaccine (7.8).

[0075] In a previous work, the inventors constructed an infectious cDNA from a batch of commercial Schwarz vaccine, a widely used MV vaccine (European Patent Application N° 02291551.6 filed on June 20, 2002). The extremities of the cDNA were engineered in order to maximize virus yield during rescue. A previously described helper cell-based rescue system was adapted by co-cultivating transfected cells on primary chick embryo fibroblasts, the cells used to produce the Schwarz vaccine. After two passages the sequence of the rescued virus was identical to that of the cDNA and of the published Schwarz sequence. Two additional transcription units (ATU) were introduced in the cDNA for cloning foreign genetic material. The immunogenicity of rescued virus was studied in mice transgenic for the CD46 MV receptor and in macaques. Antibody titers in animals inoculated with low doses of the rescued virus were identical to those obtained with commercial Schwarz MV vaccine. In contrast, the immunogenicity of a previously described Edmonston strain-derived MV clone was much lower. This new molecular clone allows producing MV vaccine without having to rely on seed stocks. The ATUs, allow producing recombinant vaccines based on an approved, efficient and worldwide used vaccine strain.

EXAMPLE 2: CONSTRUCTION OF SCHWARZ MV-WNV RECOMBINANT PLASMIDS.

1) Secreted glycoprotein E from WNV

[0076] The WNV env gene encoding the secreted form of the protein was generated by RT-PCR amplification of viral RNA purified from viral particles (WNV IS-98-ST1 strain). The specific sequence was amplified using PfuTurbo DNA polymerase (Stratagene) and specific primers that contain unique sites for subsequent cloning in pTM-MVSchw vector : MV-WNEnv5 5'-TATCGTACGATGAGAGTTGTGTTTGTCGTGCTA-3' (SEQ ID NO: 9) (BsiWI site underlined) and MV-WNEnv3 5'-ATAGCGCGCTTAGACAGCCTTCCCAACTGA-3' (SEQ ID NO: 10) (BssHII site underlined). A start and a stop codon were added at both ends of the gene. The whole sequence generated is 1380 nucleotides long (see Figure 1), including the start and the stop codons and respects the "rule of six", stipulating that the nucleotides number of MV genome must be divisible by 6 (28, 29). The Env protein thus generated contains its signal peptide in N-term (18 aa) and no transmembrane region. Thus, It represents amino acids 275-732 in WNV polyprotein and has the sequence shown in Figure 2.

2) preM plus E glycoproteins from WNV

[0077] The WNV gene encoding the preM plus E glycoproteins was generated by PCR amplification of plasmid pVL prM-E.55.1 (clone CNCM I-2732 deposited on Octobre 15, 2001). This expression plasmid encodes the pre-M and E proteins of WNV (IS-98-ST1 strain). The sequence was amplified using PfuTurbo DNA polymerase (Stratagene) and specific primers that contain unique sites for subsequent cloning in pTM-MVSchw vector : MV-WNpreME5 5'-TATCG-TACGATGCAAAAGAAAAGAGGAGGAAAG-3' (SEQ ID NO: 11) (BsiWI site underlined) and MV-WNpreME3 5'-AT-AGCGCGCTTAAGCGTGCACGTTCACGGAG-3' (SEQ ID NO: 12) (BssHII site underlined). A start and a stop codon were added at both ends of the gene. The whole sequence generated is 2076 nucleotides long (see Figure 3), including the start and the stop codons and respects the MV "rule of six". In this construct, the C-terminus part of the C protein

serves as a prM translocation signal. Both preM and E viral glycoproteins are transmembrane glycoproteins type I. It is presumed that WNV env preME expressing MV will produce and release multimeric forms of preM-E heterodimers exhibiting high immunogenic potential. The construct represents amino acids 302-789 in WNV polyprotein and has the sequence shown in Figure 4.

3. preM-E protein from Dengue type 1 virus

[0078] The Dengue virus gene encoding the preM plus E glycoproteins was generated by PCR amplification of plasmid pVL pIND/[prM+E] (clone 2) (COURAGEOT, M.-P., et al. 2000, A-glucosidase inhibitors reduce dengue virus production by affecting the initial steps of virion morphogenesis in the endoplasmic reticulum. Journal of Virology 74: 564-572). This plasmid encodes the pre-M and E glycoproteins of DEN-1 virus (strain FGA/89). The sequence was amplified using PfuTurbo DNA polymerase (Stratagene) and specific primers that contain unique sites for subsequent cloning in pTM-MVSchw vector : MV-DEN1preME5 5'-TATCGTACGATGAACAGGAGGAAAAGATCCGTG-3' (SEQ ID NO: 15) (BsiWI site underlined) and MV-DEN1preME3 5'-ATAGCGCGCTTAAACCATGACTCCTAGGTACAG-3' (SEQ ID NO: 16) (BssHII site underlined). A start and a stop codon were added at both ends of the gene. The whole sequence generated is 2040 nucleotides long (see Figure 5), including the start and the stop codons and respects the MV "rule of six". In this construct, the C-terminus part of the C protein serves as a preM translocation signal. Both preM and E viral glycoproteins are transmembrane glycoproteins type I. It is presumed that DEN-1 env expressing MV will produce and realease multimeric forms of preM-E heterodimers exhibiting high immunogenic potential. The construct represents amino acids 95-773 in DEN-1 polyprotein and has the sequence shown in Figure 6.

[0079] The same immunogens can be prepared by the same way from DEN-2, DEN-3 and DEN-4 serotypes.

4) Insertion into MV Schwarz vector

[0080] The different WNV and DEN-1 nucleotidic sequences were cloned in pCR2.1-TOPO plasmid (Invitrogen) and sequenced to check that no mutations were introduced. After BsiWI/BssHII digestion of the pCR2.1-TOPO plasmids, the DNA fragments were cloned in the pTM-MVSchw vector in ATU position 2 giving plasmids : pTM-MVSchw-EnvWNV, pTM-MVSchw-preMEwnv, and pTM-MVSchw-preMEDEN-1 according to Figure 7.

EXAMPLE 3: RECOVERY OF RECOMBINANT MVSCHW-ENVWNV, MVSCHW-PREMEWNV AND MVSCHW-NS1WNV VIRUSES.

[0081] To recover recombinant Schwarz viruses from the plasmids, we used the helper-cell-based rescue system described by Radecke et al. (11) arid modified by Parks et al. (30). Human helper cells stably expressing T7 RNA polymerase and measles N and P proteins (293-3-46 cells, a kind gift from MA Billeter) were transfected using the calcium phosphate procedure with pTM-MVSchw-EnvWNV, pTM-MVSchw-preMEwnv plasmids (5 μg) and a plasmid expressing the MV polymerase L gene (pEMC-La, 20 ng, a kind gift from MA Billeter). After overnight incubation at 37° C, the transfection medium was replaced by fresh medium and a heat shock was applied (43° C for two hours) (30). After two days of incubation at 37° C, transfected cells were transferred on a CEF cells layer and incubated at 32° C in order to avoid any adaptation of the Schwarz vaccine that was originally selected on CEF cells and is currently grown on these cells for safety considerations. Infectious virus was easily recovered between 3 and 7 days following cocultivation. Syncytia appeared occasionally in CEF, but not systematically. The recombinant viruses were also rescued by the same technique after cocultivation of transfected 293-3-46 helper cells at 37° C with primate Vero cells (african green monkey kidney). In this case, syncytia appeared systematically in all transfections after 2 days of coculture. In order to increase the yield of rescue and because these recombinant viruses will be used in mice experiments, Vero cells were used as target cells in place of the usual chick embryo fibroblasts (CEF) (European Patent Application N° 02291551.6 files on June 20, 2002). Recombinant viruses were passaged two times on Vero cells. The inventors have previously shown that two passages of the Schwarz virus on Vero cells did not change its immunogenic capacities in macaques (European Patent Application N° 02291551.6 files on June 20, 2002).

[0082] The recombinant viruses were prepared as described above and the expression of the transgene in infected cells was checked by immunofluorescence. To detect WNV Envelope glycoproteins expression, immune sera from mice resistant to WNV infection were used (International Patent Application WO 02/081741).

EXAMPLE 4: VACCINATION AGAINST WEST-NILE VIRUS

[0083] West Nile disease has recently emerged as an important mosquito-borne flavivirus infection with numerous fatal cases of human encephalitis, thus urging to develop a safe and efficient vaccine. Measles virus (MV) vaccine, a live-attenuated RNA virus, is one of the safest and most effective human vaccine developed so far. The Schwarz vaccine

strain of MV can be used as a vector to immunize against heterologous viral, thereby offering a novel and attractive vaccination strategy against West Nile virus (WNV). We evaluated the efficacy of a Schwarz measles vaccine-derived vector expressing the secreted form of the WNV envelope E glycoprotein in a mouse model. Vaccination induced high titers of specific anti-WNV neutralizing antibodies and protection from a lethal WNV challenge. Passive administration with antisera from immunized mice also provided protection, even after challenge with high doses of WNV. Example 4 is the first report that a live-attenuated recombinant measles virus provides efficient protective immunity against an heterologous viral disease. The induction of protective immunity shows that live attenuated-MV expressing the secreted form of the E glycoprotein is an effective vaccine against West Nile disease.

MATERIALS AND METHODS

[0084] Cells and virus. Vero-NK (African green monkey kidney) cells were maintained in DMEM Glutamax (Invitrogen) supplemented with 5% heat-inactivated fetal bovine serum (FBS). Helper 293-3-46 cells used for viral rescue (11) (a kind gift from M. Billeter, Zurich University) were grown in DMEM/10% FBS and supplemented with 1.2 mg of G 418 per ml. WNV strain IS-98-ST1 (GenBank accession number AF 481864) was propagated in mosquito *Aedes pseudoscutellaris* AP61 cell monolayers (13). Purification on sucrose gradients, and virus titration on AP61 cells by focus immunodetection assay (FIA) were performed as previously described (13, 27).

[0085] 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. Mouse polyclonal anti-WNV antibodies were obtained by immunization of adult BALB/c-MBT congenic mice with 10^3 FFU of IS-98-ST1 as described previously (13). The WNV-immune serum was collected one month after priming.

[0086] Construction of pTM-MVSchw-sE$_{WNV}$ plasmid. The plasmid pTM-MVSchw that contains an infectious MV cDNA corresponding to the anti-genome of the widely used Schwarz/Moraten MV vaccine strain has been reported elsewhere (10). Additional transcription units were introduced into the viral genome to turn it into a vector expressing foreign proteins. To construct pTM-MVSchw-sE$_{WNV}$, genomic RNA of WNV was extracted from highly purified IS-98-ST1 virions and reverse transcribed using Titan One-Step RT-PCR kit (Roche Molecular Biochemicals) according to the manufacturer's instructions. An RT-PCR fragment encoding the internal E translocation signal (prM-151 to prM-166) followed by the ectodomain and the stem region of the E protein (E-1 to E-441) was generated using the 5' primer MV-WNEnv5 5'-TATCGTACGATGAGAGTTGTGTTTGTCGTGCTA 3' (SEQ ID NO: 9) containing *a BsiWI* restriction site (underlined) and the 3' primer MV-WNEnv3 5'-ATAGCGCGCGCTTAGACAGCCTTCCCAACTGA-3' (SEQ ID NO: 10) containing a *BssHII* restriction site (underlined). A start and a stop codon were added at both ends of the gene. The sequence respects the «rule of six», stipulating that the nucleotides number of MV genome must be multiple of 6 (28, 29). The PCR product was directly inserted into pCR2.1-TOPO plasmid (TOPO TA cloning kit, Invitrogen) according to the manufacturer's instructions to give TOPO-sE$_{WNV}$. A 1.4-kb fragment containing truncated E protein with translocation signal sequence was excised from TOPO-sE$_{WNV}$ using *BsiWI* and *BssHII* and then inserted into *BsiWI*/*BssHII*-digested pTM-MVSchw-ATU2 which contains the additional transcription unit (ATU) between the P and M genes of Schwarz MV genome (10, 11). The resulting plasmid was designated pTM-MVSchw-sE$_{WNV}$ (named pTM-MVSchw-EnvWVN in the previous Examples). All constructs were verified by automated sequencing.

[0087] Rescue of recombinant MVSchw-sE$_{WNV}$ virus from the cloned cDNA. . Rescue of recombinant Schwarz MV from the plasmid pTM-MVSchw-sE$_{WNV}$ was performed using the helper-cell-based rescue system described by Radecke et al. (11) and modified by Parks et al. (30). Briefly, human helper cells stably expressing T7 RNA polymerase and measles N and P proteins (293-3-46 cells, a kind gift from MA Billeter, Zurich University) were transfected with 5 μg pTM-MVSchw-sE$_{WNV}$ and 0.02 μg pEMC-La expressing the MV polymerase L gene (a kind gift from MA Billeter) using the calcium phosphate procedure. After overnight incubation at 37° C, a heat shock was applied for 2 h at 43°C. After two days of incubation at 37° C, transfected cells were transferred onto a Vero cell monolayer. Vero cells were used as target cells in place of the usual chick embryo fibroblasts (CEF) in order to increase the yield of rescued virus. The inventors have previously shown that two passages of the Schwarz virus on Vero cells did not change its immunogenicity in primates (10). Syncytia that appeared after 2-3 days of coculture were transferred to 35 mm wells of Vero cells, then expanded in 75-and then 150-cm^2 flasks in DMEM/5% FBS. When syncytia reached 80-90% confluence (usually 36-48 h post-infection), the cells were scraped in a small volume of OptiMEM (invitrogen) and frozen and thawed once. After low-speed centrifugation to pellet cellular debris, the supernatant, which contained virus, was stored at -80°C. The titers of MVSchw-sE$_{WNV}$ was determined by an endpoint limit dilution assay on Vero cells. The 50% tissue culture infectious doses (TCID$_{50}$) were calculated using the Kärber method.

[0088] Radioimmunoprecipitation assay. Vero cells were starved for 1 h with DMEM without methionine and cysteine (ICN Biomedicals) and labeled 3 h with 250 μCi/ml Tran^{35}S-label (ICN Biomedicals). Cells were lysed with RIPA buffer (20 mM TrisCl, pH 8.0, 150 mM NaCl, 10 mM EDTA, 0,1%SDS, 0.5% deoxycholate, 1% Triton X-100) supplemented with a cocktail of protease inhibitors. RIP assay was performed as previously described (31). Samples were analyzed by SDS-15% PAGE under reducing conditions.

[0089] **Mice experiments.** CD46-IFNAR mice were produced as previously described (10). Adult BALB/c mice were purchased from Janvier Laboratories (Le Genest St Isle, France). Mice were housed under specific pathogen-free conditions at the Pasteur Institute. Five to 6-week-old CD46-IFNAR mice were i.p. inoculated with 10^4 or 10^6 $TCID_{50}$ of MV. Acute WNV challenge was performed by i.p. inoculation of neurovirulent WNV strain IS-98-ST1 (i.p.LD_{50} = 10) in Dulbecco's modified phosphate saline buffer (DPBS) supplemented with 0.2% bovine serum albumin (BSA) pH 7.5 (Sigma Chemical Co.). The animals were monitored daily for signs of morbidity and mortality. All experiments are approved and conducted in accordance with the guidelines of the Office Laboratory Animal Care at Pasteur Institute.

[0090] **Anti-WN vaccination test with antigenic boost.** Adult $CD46^{+/-}$ IFN-$\alpha/\beta R^{-/-}$ mice were vaccinated over a four week period with the MV-WN sE virus at a dose of 10^4 DCIP50 (which is a dose recommended for humans) and an antigenic boost was provided by purified WNV pseudo-particles that were secreted by MEF/3T3.Tet-Off/WN prME # h2 cells.

[0091] Humoral response. To evaluate the specific antibody response in serum, mice were bled via the periorbital route at different time after inoculation. Detection of anti-MV antibodies was performed by ELISA (Trinity Biotech, USA) as previously described (10). An anti-mouse antibody-HRP conjugate (Amersham) was used as the secondary antibody. The endpoint titer was calculated as the reciprocal of the last dilution giving a positive optical density value. The presence of anti-WNV antibodies was assessed by ELISA as previously described (13). Briefly, microtitration plaques were coated with 106 FFU of highly purified WNV strain IS-98-ST1 and then incubated with mouse sera dilutions. A test serum was considered positive if its optical density was twice the optical density of sera from Immunized control mice.

[0092] **Neutralization assay.** anti-WNV neutralizing antibodies were detected by a FRNT test. Sera from each mouse group were pooled and heat-inactivated at 56°C for 30 min. Vero cells were seeded into 12-well plate (1.5 x 10^5 cells/well) for 24 h. Mouse serum samples were serially diluted in MEM Glutamax/2% FBS. Dilutions (0.1 ml) were Incubated at 37°C for 2 h and under gentle agitation with an equal volume of WNV strain IS-98-ST1 containing ~100 FFU. Remaining infectivity was then assayed on Vero cell monolayer overlaid with MEM Glutamax/2%FBS containing 0.8% ($^w/_v$) carboxy methyl cellulose (BDH). After 2 days of incubation at 37°C with 5% CO_2, FIA was performed with anti-WNV HMAF as previously described (27). The highest serum dilution tested that reduced the number of FFU by at least 90% ($FRNT_{90}$) was considered the end-point titer.

[0093] **Passive transfer of immune sera.** Pooled immune sera were transferred into 6-week-old female BALB/c mice intraperitoneally. Mice received injection of 0.1 ml of serial dilutions of pooled serum samples in DPBS/0.2%BSA one day before WNV inoculation. The challenged mice were observed for more than 3 weeks.

DISCUSSION OF THE RESULTS

[0094] Since its introduction into the United States in 1999, West Nile virus (WNV) infection has been recognized as one of the most serious mosquito-borne disease in the Western Hemisphere, causing severe neurological disease (meningoencephalitis and poliomyelitis-like syndrome) in humans. (3). Within the last 4 years; WNV had spread through North America, Central America and the Caribbean (1, 2). It is presumed that it will reach South America in the coming years. Since 2002, the US outbreaks were characterized by an apparent increase in human disease severity with 13,000 cases and 500 deaths. Although mosquito-borne transmission of WNV predominates, WNV is also transmitted by blood transfusion, organ donations and transplacentaly to the fetus (3). Prevention of West Nile encephalitis is a new public health priority and it is imperative that a vaccine be developed (3, 4, 5). No vaccine has been approved for human use so far.

[0095] Because WNV can be transmitted across species, there is an urgent need to develop preventive strategies for humans. A rational approach should be to confer a long-term immunity in large groups of individuals, and to boost this immunity in case of WNV outbreaks. Measles virus (MV) vaccine can now be used as a vector to immunize against heterologous viral diseases, thereby offering a novel and attractive vaccination strategy against WNV. We have recently tested this vector against HIV infection (6). MV vaccine, a live-attenuated RNA virus, is one of the safest and most effective human vaccine developed so far. It induces a very efficient, life-long immunity after a single or two injections (7, 8). The MV genome is very stable and reversion of vaccine strains to pathogenicity has never been observed. The Schwarz MV strain is used in two widely used measles vaccines, Attenuavax (Merck and Co. Inc., West Point, USA) and Rouvax (Aventis Pasteur, Marcy l'Etoile, France), and in the combined measles, mumps, and rubella vaccine (MMR) (9). We have recently generated an infectious cDNA for this strain (10) and introduced additional transcription units (ATU) into it for cloning foreign genes, based on the work of Radecke et al. (11). The vaccine rescued from the molecular clone was as immunogenic as the parental vaccine in primates and mice susceptible to MV infection. Thus, this approved and widely used MV vaccine can be used as a vector to immunize individuals simultaneously against measles and other infectious diseases.

[0096] WNV is a single-stranded RNA virus of the *Flaviviridae* family, genus flavivirus, within the Japanese encephalitis antigenic complex (2, 3). The virion is composed of three structural proteins, designated C (core protein), M (membrane protein) and E (envelope protein). Protein E, which is exposed on the surface of the virion, is responsible for virus attachment and virus-specific membrane fusion. Because the E glycoprotein can potentially serve as a major protective

immunogen for a WNV vaccine (12), the inventors introduced the WNV cDNA encoding the carboxyl-terminally truncated E glycoprotein lacking the transmembrane-anchoring region (residues E-1 to E-441, designated sE_{WNV} hereinafter) of IS-98-ST1 strain (13) into the infectious cDNA for the Schwarz MV vaccine (10) (Fig. 8A). WNV strain IS-98-ST1 has the same neuropathologic properties than the new variant designated Isr98/NY99 that has been responsible for the recent WNV outbreaks in North America and Middle East (13). The WNV sequence was introduced in an ATU located between the phosphoprotein (P) and matrix (M) genes in the MV genome. The recombinant MVSchw-sE_{WNV} virus was produced after transfection of the corresponding plasmid into human helper cells allowing the rescue of negative-stranded RNA paramyxoviruses (11), then propagation in Vero cell cultures. The growth of MVschw-sE_{WNV} in Vero cells was only slightly delayed as compared to that of standard Schwarz MV (MVschw) (Fig. 8B). After 60 h of infection, the yield of MVSchw-sE_{WNV} was comparable to that of MVschw. The expression of sE_{WNV} in MVschw-sE_{WNV}-infected Vero cells was demonstrated by immunofluorescence and radioimmunoprecipitation (RIP) assays (Fig. 8C, D). At 40 h post-infection, the cell surface of MVSchw-sE_{WNV}-induced syncitia was clearly visualized by anti-WNV immune serum, indicating that sE_{WNV} is transported along the compartments of the secretory pathway (Fig. 8C). RIP analysis revealed that anti-WNV antibodies recognized sE_{WNV} that migrated faster than authentic E glycoprotein (Fig. 8D). Interestingly, sE_{WNV} was detected in the supernatants of MVschw-sE_{WNV}-infected Vero cells at 40 h post-infection (Fig. 8D, panel Supernatants/ MVSchw-sE_{WNV}, lane α-WNV). Thus, MVSchw-sE_{WNV} expresses a recombinant E glycoprotein which is secreted efficiently. Immunoblots confirmed that sE_{WNV} accumulated in the culture medium of MVSchw-sE_{WNV}-infected Vero cells (data not shown).

[0097] Genetically modified mice expressing the human CD46 MV receptor and lacking the interferon α/β receptor (6, 14) ($CD46^{+/-}$ IFN-α/β $R^{-/-}$, abbreviated CD46-IFNAR) that are susceptible to MV (14) were used to assess the immune response induced by MVSchw-sE_{WNV}. These mice deficient in IFN-α/β response raise cellular and humoral immune responses similar to those of competent mice (6, 10, 15, 16). Two groups of six CD46-IFNAR mice were inoculated intraperitoneally (i.p.) with either 10^4 or 10^6 tissue culture infective doses ($TCID_{50}$) of MVSchw-sE_{WNV}. Each group was boosted using the same dose 1 month after the first immunization. As a control, CD46-IFNAR mice were immunized with 10^6 $TCID_{50}$ of "empty" MVSchw. One month after the first immunization, specific anti-MV antibodies were detected in immune sera from mice inoculated with either MVSchw or MVSchw-sE_{WNV} (Table 1). Mice that received either dose of MVSchw-sE_{WNV} displayed specific anti-WNV antibodies at a dilution of 1:3,000. One month after boosting, the titers of anti-WNV antibodies had reached 1:30,000 to 1:200,000 (Table 1) and were highly reactive with the WNV E glycoprotein (Fig. 9). No anti-WNV antibodies were detected in the sera of any control mice (Table 1 and Fig. 9). These results show that one injection of MVSchw-sE_{WNV} induces anti-WNV antibodies, and that boosting one month after priming increases their titers 10 to 60 times.

[0098] Anti-WNV neutralizing activity was measured in MVSchw-sE_{WNV}-immune sera using a focus reduction test ($FRNT_{90}$) (Table 1). As a positive control, the WNV-immune serum from immunized BALB/c-MBT congenic mice (13) gave a $FRNT_{90}$ titer of 50. The immune sera from CD46-IFNAR mice inoculated with "empty" MVSchw had not detectable neutralizing activity. Immunized CD46-IFNAR mice which received 10^4 or 10^6 $TCID_{50}$ of MVSchw-sE_{WNV} raised neutralizing antibodies with similar $FRNT_{90}$ titers, and boosting increased their titers from 10 to 200-300. These data show that mice twice inoculated with the recombinant live-attenuated MV encoding the secreted form of the IS-98-ST1 E glycoprotein had high levels of anti-WNV antibody with neutralizing activity, regardless of the injected dose.

[0099] Because antibody-mediated immunity may be critical to protect against WNV infection (17, 18), the inventors examined if the passive transfer of sera from MVSchw-sE_{WNV}-immunized mice can protect adult BALB/c mice from WNV infection (Table 2). Groups of six 6-week-old BALB/c mice received i.p. various amounts of pooled immune sera from MVSchw-sE_{WNV}-immunized CD46-IFNAR mice collected one month after priming or boosting. One day later, the mice were challenged with 10 times the i.p. 50% lethal dose (LD_{50}) of WNV strain IS-98-ST1 (13, 19). As a positive control, BALB/c mice that received as little as 2 μl of the WNV-immune serum were protected from the challenge (Table 2). In contrast, all mice that received 2 μl of the non-immune mouse serum or serum from "empty" MVSchw-immunized mice died within 11-12 days. Protective passive immunity was observed in all BALB/c mice following transfer of 2 μl of pooled sera from CD46-IFNAR mice immunized once with 10^6 $TCID_{50}$ of MVSchw-sE_{WNV}. As little as 1 μl of this antisera induced 66% protection. Passive transfer of sera collected one month after a single immunization with 10^4 $TCID_{50}$ induced a survival rate of 50 %. Remarkably, the administration of 1 μl of MVSchw-sE_{WNV}-immune sera collected 1 month after boosting induced 100% protection. These results indicate that a single injection of 10^6 $TCID_{50}$ or two injections of 10^4 $TCID_{50}$ of MVSchw-sE_{WNV} elicited protective humoral response. Because the amount of flavivirus inoculated during mosquito feeding is probably in the order of 10^2 to 10^4 infectious virus particles (1), we assessed the capacity of MVSchw-sE_{WN}-immune sera to protect against a range of 10^2 to 10^5 focus forming units (FFU) of WNV strain IS-98-ST1. Groups of six BALB/c mice were passively immunized with 2 μl of pooled immune sera collected from CD46-IFNAR mice twice inoculated with 10^4 $TCID_{50}$ of MVSchw-sE_{WNV} (Table 2). Survival rates of 85-100% were observed in mice that received the MVSchw-sE_{WNV}-immune serum, regardless the lethal doses of IS-98-ST1 (10 to 10,000 i.p. LD_{50}). These data are consistent with the finding that humoral response plays a critical role in protection against WNV infection.

[0100] Mice which are completely unresponsive to IFN-α/β are highly susceptible to encephalitic flaviviruses (19, 20).

Indeed, the inventors previously showed that WNV infection of CD46-IFNAR mice was lethal within 3 days instead of 11 days in competent mice (19). To assess whether the immunity induced by MVSchw-sE_{WNV} could protect these compromised animals from WNV infection, three CD46-IFNAR mice from the group that had received two injections of MVSchw-sE_{WNV} (10^6 $TCID_{50}$), were i.p. inoculated with 100 FFU of IS-98-ST1 one month after the boost Mice inoculated with "empty" MVSchw were used as controls. The mice that had received MVSchw-sE_{WNV} survived the WNV challenge while control mice died within 3 days. MVSchw-sE_{WNV}-immunized mice were bled 3 weeks after challenge. The $FRNT_{90}$ antibody response (titer ~ 100) was comparable to the pre-challenge response. Notably, post-challenge immune sera did not react with WNV nonstructural proteins such as NS3 and NS5 as shown by RIP assay (Fig. 9, panel MVSchw-sE_{WNV}, lane 10^6 $TCID_{50}$, day 20, p.c.), suggesting that no viral replication occurred after challenge with WNV. These data show that immunizing with MVSchw-sE_{WNV} prevented WNV infection in highly susceptible animals.

[0101] The present Example shows for the first time that a live-attenuated measles vector derived from the Schwarz MV vaccine can induce a protective immunity against an heterologous lethal pathogen. These data constitute also the proof of concept that a live-attenuated Schwarz measles vaccine engineered to express the secreted form of the WNV E glycoprotein can be used as a vaccine to present West Nile disease in humans. The MV vaccine vector offers several advantages over other existing viral vectors. The Schwarz MV vaccine has been used on billions of people since the sixties and shown to be safe and efficacious. It is easily produced on a large scale in most countries and can be distributed at low cost. The MV genome is very stable and reversion to pathogenicity has never been observed (8). Moreover, MV replicates exclusively in the cytoplasm, ruling out the possibility of integration in host DNA. The MV vector has been shown to express a variety of genes, or combinations of genes, of large size over more than twelve passages (6, 16, 21, 22, 23, 24). This stability is likely due to the fact that there is little constraint on genome size for pleomorphic viruses with a helical nucleocapsid. Unlike chimeric viral vectors, the recombinant MV vector is an authentic MV expressing an additional gene. This greatly reduces the risk of changing the tropism and the pathogenicity of the original vaccine. It reduces also the risk of recombination.

[0102] The recombinant MV-WNV vaccine according to a preferred embodiment of the present invention is a promising live-attenuated vector to mass immunize children and adolescents against both measles and West Nile diseases. Although the existence of an anti-MV immunity in nearly the entire adult human population appears to restrict its use to infants, an already worthy goal, recent studies demonstrated that revaccinating already immunized children results in a boost of anti-MV antibodies (25, 26). These and other studies (Ann Arvin) demonstrated that the presence of passive MV pre-immunity (maternal antibodies) does not circumvent the replication of attenuated MV after a second injection. This opens the possibility of using the live-attenuated MV-derived vector to immunize adults. Indeed, the inventors reported that a MV-HIV recombinant virus induced anti-HIV neutralizing antibodies in mice and macaques even in the presence of pre-existing anti-MV immunity (6). Because of cross-species transmission, it is feared that WNV becomes a recurrent zoonosis with repeated seasonal outbreaks in humans. The inventors propose that MVSchw-sE_{WNV} could be used to Induce long-term memory immunity in large groups of children and adults, and to boost this immunity in case of West Nile disease outbreak.

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TABLE 1. Antibody response of CD46-IFNAR mice to intraperitoneal inoculation of MVSchw-sE$_{WNV}$

Immunizing virus	MV-specific Ab titer[4]	WN-specific Ab titer[4]	WN-specific FRNT$_{90}$ [5]
WNV[1] (10^3 FFU)	NT	10,000	50
MVSchw [2] (10^6 TCID$_{50}$)	30,000	<10	<10
MVSchw-sE$_{WNV}$[2] (10^4 TCID$_{50}$)	15,000	3,000	10
MVSchw-sE$_{WNV}$[2] (10^6 TCID$_{50}$)	25,000	3,000	10
2 x MVSchw-sE$_{WNV}$[3] (10^4 TCID$_{50}$)	90,000	30,000	200
2 x MVSchw-sE$_{WNV}$[3] (10^6 TCID$_{50}$)	140,000	200,000	300

[1]BALB/c-MBT congenic mice were i.p. inoculated with WNV strain IS-98-ST1.
[2]Virus was given i.p. to CD46-IFNAR mice.
[3]Virus was given i.p. twice at 1 month of interval.
[4]Determined by ELISA on pooled heat-inactivated sera.
[5]The highest serum dilution that reduced the number of FFU of WNV by at least 90%. NT: not tested

TABLE 2. Protective ability of the MVSchw-sE$_{WNV}$-immune serum

Material used for immunization	Volume of sera transferred[1] (□l)	WNV[2] (FFU)	Protection (no. surviving/ no. tested)	M.D.O.D[3] (day ± S.D.)
Controls				
DPBS	10	100	0/6	11.5 ± 1.5
WNV[4]	10	100	6/6	-
	2	100	5/6	20
MVSchw[5]	2	100	0/6	12.0 ± 1.5
MVSchw-sE $_{WNV}$[6]	2	100	6/6	-
10^8 TCID50 (day 30)	1	100	4/6	11.0±1.5
10^4 TCID50 (day 30)	10	100	3/6	10.5 ± 2.0
10^4 TCID50 (day 60)	1	100	6/6	-
	2	100	5/6	11
	2	1,000	6/6	-
	2	10,000	5/6	10

(continued)

Material used for immunization	Volume of sera transferred[1] (□l)	WNV[2] (FFU)	Protection (no. surviving/ no. tested)	M.D.O.D[3] (day ± S.D.)
	2	100,000	5/6	11

[1]BALB/c mice received 0.1 ml of DPBS containing the indicated amount of pooled sera.
[2]Mice were challenged with WNV strain IS-98-ST1 one day after passive transfer.
[3]Mean day of death ± standard deviation.
[4]Immune sera from resistant BALB/c-MBT congenic mice (13) inoculated with 10^3 FFU of IS-98-ST1 WNV.
[5]Immune sera from CD46-IFNAR mice collected 30 days after inoculation of MVSchw (10^6 $TCID_{50}$).
[6]Immune sera from CD46-IFNAR mice were collected 30 days after 1 injection or 60 days after 2 injections of MVSchw-sE_{WNV}.

SEQUENCE LISTING

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gttggagggg tgttcacctc agttgggaag gctgtccatc aagtgttcgg aggagcattc    1920
cgctcactgt tcggaggcat gtcctggata acgcaaggat tgctgggggc tctcctgttg    1980
tggatgggca tcaatgctcg tgataggtcc atagctctca cgtttctcgc agttggagga    2040
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<210> 3
<211> 1110
<212> DNA
<213> West-Nile Virus

<400> 3

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atgaggtcca tagctctcac gtttctcgca gttggaggag ttctgctctt cctctccgtg      60
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agtggagtgt tcatacacaa tgatgtggag gcttggatgg accggtacaa gtattaccct     180
gaaacgccac aaggcctagc caagatcatt cagaaagctc ataaggaagg agtgtgcggt     240
ctacgatcag tttccagact ggagcatcaa atgtgggaag cagtgaagga cgagctgaac     300
actcttttga aggagaatgg tgtggacctt agtgtcgtgg ttgagaaaca ggagggaatg     360
tacaagtcag cacctaaacg cctcaccgcc accacggaaa aattggaaat tggctggaag     420
gcctggggaa agagtatttt atttgcacca gaactcgcca acaacacctt tgtggttgat     480
ggtccggaga ccaaggaatg tccgactcag aatcgcgctt ggaatagctt agaagtggag     540
gattttggat ttggtctcac cagcactcgg atgttcctga aggtcagaga gagcaacaca     600
actgaatgtg actcgaagat cattggaacg gctgtcaaga acaacttggc gatccacagt     660
gacctgtcct attggattga aagcaggctc aatgatacgt ggaagcttga aagggcagtt     720
ctgggtgaag tcaaatcatg tacgtggcct gagacgcata ccttgtgggg cgatggaatc     780
cttgagagtg acttgataat accagtcaca ctggcgggac cacgaagcaa tcacaatcgg     840
agacctgggt acaagacaca aaaccagggc ccatgggacg aaggccgggt agagattgac     900
ttcgattact gcccaggaac tacggtcacc ctgagtgaga gctgcggaca ccgtggacct     960
gccactcgca ccaccacaga gagcggaaag ttgataacag attggtgctg caggagctgc    1020
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ccacagagac atgatgaaaa gacctaatga                                     1110
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<210> 4

<211> 2040
<212> DNA
<213> Dengue type-1 virus

<400> 4

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aagtcactct tgttcaagac ctctgcaggt gtcaatatgt gcactctcat tgcgatggat     180
ttgggagagt tatgtgagga cacaatgact tacaaatgcc cccggatcac tgaggcggaa     240
ccagatgacg ttgactgctg gtgcaatgcc acagacacat gggtgaccta tgggacgtgt     300
tctcaaaccg gtgaacaccg acgagacaaa cgttccgtgg cactggcccc acacgtggga     360
cttggtctag aaacaagaac cgaaacatgg atgtcctctg aaggcgcctg gaaacaaata     420
caaaaagtgg agacttgggc tttgagacac ccaggattca cggtgatagc tctttttta      480
gcacatgcca taggaacatc catcactcag aaagggatca ttttcattct gctgatgctg     540
gtaacaccat caatggccat gcgatgcgtg ggaataggca acagagactt cgttgaagga     600
ctgtcaggag caacgtgggt ggacgtggta ttggagcatg gaagctgcgt caccaccatg     660
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acccagcatg gaactgtcct agtgcaggtt aaatacgaag gaacagatgc gccatgcaag    1560
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gagagctaca tcatagtagg ggcaggtgaa aaagctttga aactaagctg gttcaagaaa    1740
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ctgggagaca ccgcatggga cttcggctct ataggaggag tgtttacgtc tgtgggaaaa    1860
ttggtacacc aggtttttgg aaccgcatac ggggtcctgt tcagcggcgt ttcttggacc    1920
atgaaaatag gaatagggat cttgctgaca tggttgggat taaattcaag gagcgcgtcg    1980
ctttcgatga cgtgcattgc agttggcatg gttacactgt acctaggagt catggtttaa    2040
```

<210> 5
<211> 459
<212> PRT
<213> West-Nile virus

<400> 5

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Met Arg Val Val Phe Val Val Leu Leu Leu Leu Val Ala Pro Ala
                5                  10                  15

Tyr Ser Phe Asn Cys Leu Gly Met Ser Asn Arg Asp Phe Leu Glu
            20                  25                  30

Gly Val Ser Gly Ala Thr Trp Val Asp Leu Val Leu Glu Gly Asp
        35                  40                  45

Ser Cys Val Thr Ile Met Ser Lys Asp Lys Pro Thr Ile Asp Val
    50                  55                  60

Lys Met Met Asn Met Glu Ala Val Asn Leu Ala Glu Val Arg Ser
65                  70                  75

Tyr Cys Tyr Leu Ala Thr Val Ser Asp Leu Ser Thr Lys Ala Ala
            80                  85                  90

Cys Pro Thr Met Gly Glu Ala His Asn Asp Lys Arg Ala Asp Pro
```

95 100 105

Ala Phe Val Cys Arg Gln Gly Val Val Asp Arg Gly Trp Gly Asn
110 115 120

Gly Cys Gly Leu Phe Gly Lys Gly Ser Ile Asp Thr Cys Ala Lys
125 130 135

Phe Ala Cys Ser Thr Lys Ala Ile Gly Arg Thr Ile Leu Lys Glu
140 145 150

Asn Ile Lys Tyr Glu Val Ala Ile Phe Val His Gly Pro Thr Thr
155 160 165

Val Glu Ser His Gly Asn Tyr Ser Thr Gln Val Gly Ala Thr Gln
170 175 180

Ala Gly Arg Phe Ser Ile Thr Pro Ala Ala Pro Ser Tyr Thr Leu
185 190 195

Lys Leu Gly Glu Tyr Gly Glu Val Thr Val Asp Cys Glu Pro Arg
200 205 210

Ser Gly Ile Asp Thr Asn Ala Tyr Tyr Val Met Thr Val Gly Thr
215 220 225

Lys Thr Phe Leu Val His Arg Glu Trp Phe Met Asp Leu Asn Leu
230 235 240

Pro Trp Ser Ser Ala Gly Ser Thr Val Trp Arg Asn Arg Glu Thr
245 250 255

Leu Met Glu Phe Glu Glu Pro His Ala Thr Lys Gln Ser Val Ile
260 265 270

Ala Leu Gly Ser Gln Glu Gly Ala Leu His Gln Ala Leu Ala Gly
275 280 285

Ala Ile Pro Val Glu Phe Ser Ser Asn Thr Val Lys Leu Thr Ser
290 295 300

Gly His Leu Lys Cys Arg Val Lys Met Glu Lys Leu Gln Leu Lys
305 310 315

Gly Thr Thr Tyr Gly Val Cys Ser Lys Ala Phe Lys Phe Leu Gly
320 325 330

Thr Pro Ala Asp Thr Gly His Gly Thr Val Val Leu Glu Leu Gln
335 340 345

Tyr Thr Gly Thr Asp Gly Pro Cys Lys Val Pro Ile Ser Ser Val
350 355 360

Ala Ser Leu Asn Asp Leu Thr Pro Val Gly Arg Leu Val Thr Val
365 370 375

Asn Pro Phe Val Ser Val Ala Thr Ala Asn Ala Lys Val Leu Ile
380 385 390

Glu Leu Glu Pro Pro Phe Gly Asp Ser Tyr Ile Val Val Gly Arg
395 400 405

Gly Glu Gln Gln Ile Asn His His Trp His Lys Ser Gly Ser Ser
410 415 420

```
Ile Gly Lys Ala Phe Thr Thr Thr Leu Lys Gly Ala Gln Arg Leu
                425                 430                 435

Ala Ala Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val Gly Gly
                440                 445                 450

Val Phe Thr Ser Val Gly Lys Ala Val
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```

<210> 6
<211> 691
<212> PRT
<213> West-Nile virus

<400> 6

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Met Gln Lys Lys Arg Gly Gly Lys Thr Gly Ile Ala Val Met Ile
 5                  10                  15

Gly Leu Ile Ala Ser Val Gly Ala Val Thr Leu Ser Asn Phe Gln
            20                  25                  30

Gly Lys Val Met Met Thr Val Asn Ala Thr Asp Val Thr Asp Val
        35                  40                  45

Ile Thr Ile Pro Thr Ala Ala Gly Lys Asn Leu Cys Ile Val Arg
    50                  55                  60

Ala Met Asp Val Gly Tyr Met Cys Asp Asp Thr Ile Thr Tyr Glu
65                  70                  75

Cys Pro Val Leu Ser Ala Gly Asn Asp Pro Glu Asp Ile Asp Cys
            80                  85                  90

Trp Cys Thr Lys Ser Ala Val Tyr Val Arg Tyr Gly Arg Cys Thr
        95                  100                 105

Lys Thr Arg His Ser Arg Arg Ser Arg Arg Ser Leu Thr Val Gln
    110                 115                 120

Thr His Gly Glu Ser Thr Leu Ala Asn Lys Lys Gly Ala Trp Met
125                 130                 135

Asp Ser Thr Lys Ala Thr Arg Tyr Leu Val Lys Thr Glu Ser Trp
            140                 145                 150

Ile Leu Arg Asn Pro Gly Tyr Ala Leu Val Ala Ala Val Ile Gly
        155                 160                 165

Trp Met Leu Gly Ser Asn Thr Met Gln Arg Val Val Phe Val Val
    170                 175                 180

Leu Leu Leu Leu Val Ala Pro Ala Tyr Ser Phe Asn Cys Leu Gly
185                 190                 195

Met Ser Asn Arg Asp Phe Leu Glu Gly Val Ser Gly Ala Thr Trp
            200                 205                 210

Val Asp Leu Val Leu Glu Gly Asp Ser Cys Val Thr Ile Met Ser
        215                 220                 225

Lys Asp Lys Pro Thr Ile Asp Val Lys Met Met Asn Met Glu Ala
    230                 235                 240
```

```
Val Asn Leu Ala Glu Val Arg Ser Tyr Cys Tyr Leu Ala Thr Val
245                 250                 255

Ser Asp Leu Ser Thr Lys Ala Ala Cys Pro Thr Met Gly Glu Ala
260                 265                 270

His Asn Asp Lys Arg Ala Asp Pro Ala Phe Val Cys Arg Gln Gly
275                 280                 285

Val Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys
290                 295                 300

Gly Ser Ile Asp Thr Cys Ala Lys Phe Ala Cys Ser Thr Lys Ala
305                 310                 315

Ile Gly Arg Thr Ile Leu Lys Glu Asn Ile Lys Tyr Glu Val Ala
320                 325                 330

Ile Phe Val His Gly Pro Thr Thr Val Glu Ser His Gly Asn Tyr
335                 340                 345

Ser Thr Gln Val Gly Ala Thr Gln Ala Gly Arg Phe Ser Ile Thr
350                 355                 360

Pro Ala Ala Pro Ser Tyr Thr Leu Lys Leu Gly Glu Tyr Gly Glu
365                 370                 375

Val Thr Val Asp Cys Glu Pro Arg Ser Gly Ile Asp Thr Asn Ala
380                 385                 390

Tyr Tyr Val Met Thr Val Gly Thr Lys Thr Phe Leu Val His Arg
395                 400                 405

Glu Trp Phe Met Asp Leu Asn Leu Pro Trp Ser Ser Ala Gly Ser
410                 415                 420

Thr Val Trp Arg Asn Arg Glu Thr Leu Met Glu Phe Glu Glu Pro
425                 430                 435

His Ala Thr Lys Gln Ser Val Ile Ala Leu Gly Ser Gln Glu Gly
440                 445                 450

Ala Leu His Gln Ala Leu Ala Gly Ala Ile Pro Val Glu Phe Ser
455                 460                 465

Ser Asn Thr Val Lys Leu Thr Ser Gly His Leu Lys Cys Arg Val
470                 475                 480

Lys Met Glu Lys Leu Gln Leu Lys Gly Thr Thr Tyr Gly Val Cys
485                 490                 495

Ser Lys Ala Phe Lys Phe Leu Gly Thr Pro Ala Asp Thr Gly His
500                 505                 510

Gly Thr Val Val Leu Glu Leu Gln Tyr Thr Gly Thr Asp Gly Pro
515                 520                 525

Cys Lys Val Pro Ile Ser Ser Val Ala Ser Leu Asn Asp Leu Thr
530                 535                 540

Pro Val Gly Arg Leu Val Thr Val Asn Pro Phe Val Ser Val Ala
545                 550                 555

Thr Ala Asn Ala Lys Val Leu Ile Glu Leu Glu Pro Pro Phe Gly
```

```
                    560                 565                 570

Asp Ser Tyr Ile Val Val Gly Arg Gly Glu Gln Gln Ile Asn His
                    575                 580                 585

His Trp His Lys Ser Gly Ser Ser Ile Gly Lys Ala Phe Thr Thr
                    590                 595                 600

Thr Leu Lys Gly Ala Gln Arg Leu Ala Ala Leu Gly Asp Thr Ala
                    605                 610                 615

Trp Asp Phe Gly Ser Val Gly Gly Val Phe Thr Ser Val Gly Lys
                    620                 625                 630

Ala Val His Gln Val Phe Gly Gly Ala Phe Arg Ser Leu Phe Gly
                    635                 640                 645

Gly Met Ser Trp Ile Thr Gln Gly Leu Leu Gly Ala Leu Leu Leu
                    650                 655                 660

Trp Met Gly Ile Asn Ala Arg Asp Arg Ser Ile Ala Leu Thr Phe
                    665                 670                 675

Leu Ala Val Gly Gly Val Leu Leu Phe Leu Ser Val Asn Val His
                    680                 685                 690

Ala
```

<210> 7
<211> 368
<212> PRT
<213> West-Nile virus

<400> 7

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Met Arg Ser Ile Ala Leu Thr Phe Leu Ala Val Gly Gly Val Leu
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Leu Phe Leu Ser Val Asn Val His Ala Asp Thr Gly Cys Ala Ile
            20                  25                  30

Asp Ile Ser Arg Gln Glu Leu Arg Cys Gly Ser Gly Val Phe Ile
        35                  40                  45

His Asn Asp Val Glu Ala Trp Met Asp Arg Tyr Lys Tyr Tyr Pro
    50                  55                  60

Glu Thr Pro Gln Gly Leu Ala Lys Ile Ile Gln Lys Ala His Lys
65                  70                  75

Glu Gly Val Cys Gly Leu Arg Ser Val Ser Arg Leu Glu His Gln
                80                  85                  90

Met Trp Glu Ala Val Lys Asp Glu Leu Asn Thr Leu Leu Lys Glu
            95                  100                 105

Asn Gly Val Asp Leu Ser Val Val Val Glu Lys Gln Glu Gly Met
        110                 115                 120

Tyr Lys Ser Ala Pro Lys Arg Leu Thr Ala Thr Thr Glu Lys Leu
    125                 130                 135

Glu Ile Gly Trp Lys Ala Trp Gly Lys Ser Ile Leu Phe Ala Pro
```

```
                    140                 145                 150

Glu Leu Ala Asn Asn Thr Phe Val Val Asp Gly Pro Glu Thr Lys
                    155                 160                 165

Glu Cys Pro Thr Gln Asn Arg Ala Trp Asn Ser Leu Glu Val Glu
                    170                 175                 180

Asp Phe Gly Phe Gly Leu Thr Ser Thr Arg Met Phe Leu Lys Val
                    185                 190                 195

Arg Glu Ser Asn Thr Thr Glu Cys Asp Ser Lys Ile Ile Gly Thr
                    200                 205                 210

Ala Val Lys Asn Asn Leu Ala Ile His Ser Asp Leu Ser Tyr Trp
                    215                 220                 225

Ile Glu Ser Arg Leu Asn Asp Thr Trp Lys Leu Glu Arg Ala Val
                    230                 235                 240

Leu Gly Glu Val Lys Ser Cys Thr Trp Pro Glu Thr His Thr Leu
                    245                 250                 255

Trp Gly Asp Gly Ile Leu Glu Ser Asp Leu Ile Ile Pro Val Thr
                    260                 265                 270

Leu Ala Gly Pro Arg Ser Asn His Asn Arg Arg Pro Gly Tyr Lys
                    275                 280                 285

Thr Gln Asn Gln Gly Pro Trp Asp Glu Gly Arg Val Glu Ile Asp
                    290                 295                 300

Phe Asp Tyr Cys Pro Gly Thr Thr Val Thr Leu Ser Glu Ser Cys
                    305                 310                 315

Gly His Arg Gly Pro Ala Thr Arg Thr Thr Thr Glu Ser Gly Lys
                    320                 325                 330

Leu Ile Thr Asp Trp Cys Cys Arg Ser Cys Thr Leu Pro Pro Leu
                    335                 340                 345

Arg Tyr Gln Thr Asp Ser Gly Cys Trp Tyr Gly Met Glu Ile Arg
                    350                 355                 360

Pro Gln Arg His Asp Glu Lys Thr
                    365
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<210> 8
<211> 679
<212> PRT
<213> Dengue type-1 virus

<400> 8

Met Asn Arg Arg Lys Arg Ser Val Thr Met Leu Leu Met Leu Leu
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Pro Thr Val Leu Ala Phe His Leu Thr Thr Arg Gly Gly Glu Pro
20 25 30

His Met Ile Val Ser Lys Gln Glu Arg Gly Lys Ser Leu Leu Phe
35 40 45

```
Lys Thr Ser Ala Gly Val Asn Met Cys Thr Leu Ile Ala Met Asp
                50                  55                  60

Leu Gly Glu Leu Cys Glu Asp Thr Met Thr Tyr Lys Cys Pro Arg
                65                  70                  75

Ile Thr Glu Ala Glu Pro Asp Asp Val Asp Cys Trp Cys Asn Ala
                80                  85                  90

Thr Asp Thr Trp Val Thr Tyr Gly Thr Cys Ser Gln Thr Gly Glu
                95                  100                 105

His Arg Arg Asp Lys Arg Ser Val Ala Leu Ala Pro His Val Gly
                110                 115                 120

Leu Gly Leu Glu Thr Arg Thr Glu Thr Trp Met Ser Ser Glu Gly
                125                 130                 135

Ala Trp Lys Gln Ile Gln Lys Val Glu Thr Trp Ala Leu Arg His
                140                 145                 150

Pro Gly Phe Thr Val Ile Ala Leu Phe Leu Ala His Ala Ile Gly
                155                 160                 165

Thr Ser Ile Thr Gln Lys Gly Ile Ile Phe Ile Leu Leu Met Leu
                170                 175                 180

Val Thr Pro Ser Met Ala Met Arg Cys Val Gly Ile Gly Asn Arg
                185                 190                 195

Asp Phe Val Glu Gly Leu Ser Gly Ala Thr Trp Val Asp Val Val
                200                 205                 210

Leu Glu His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro
                215                 220                 225

Thr Leu Asp Ile Glu Leu Leu Lys Thr Glu Val Thr Asn Pro Ala
                230                 235                 240

Val Leu Arg Lys Leu Cys Ile Glu Ala Lys Ile Ser Asn Thr Thr
                245                 250                 255

Thr Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala Thr Leu Val Glu
                260                 265                 270

Glu Gln Asp Ala Asn Phe Val Cys Arg Arg Thr Val Val Asp Arg
                275                 280                 285

Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser Leu Leu
                290                 295                 300

Thr Cys Ala Lys Phe Lys Cys Val Thr Lys Leu Glu Gly Lys Ile
                305                 310                 315

Val Gln Tyr Glu Asn Leu Lys Tyr Ser Val Ile Val Thr Val His
                320                 325                 330

Thr Gly Asp Gln His Gln Val Gly Asn Glu Thr Thr Glu His Gly
                335                 340                 345

Thr Ile Ala Thr Ile Thr Pro Gln Ala Pro Thr Ser Glu Ile Gln
                350                 355                 360

Leu Thr Asp Tyr Gly Thr Leu Thr Leu Asp Cys Ser Pro Arg Thr
                365                 370                 375
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Gly Leu Asp Phe Asn Glu Val Val Leu Leu Thr Met Lys Glu Lys
380 385 390

Ser Trp Leu Val His Lys Gln Trp Phe Leu Asp Leu Pro Leu Pro
395 400 405

Trp Thr Ser Gly Ala Ser Thr Ser Gln Glu Thr Trp Asn Arg Gln
410 415 420

Asp Leu Leu Val Thr Phe Lys Thr Ala His Ala Lys Lys Gln Glu
425 430 435

Val Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu
440 445 450

Thr Gly Ala Thr Glu Ile Gln Thr Ser Gly Thr Thr Thr Ile Phe
455 460 465

Ala Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Thr Leu
470 475 480

Lys Gly Met Ser Tyr Val Met Cys Thr Gly Ser Phe Lys Leu Glu
485 490 495

Lys Glu Val Ala Glu Thr Gln His Gly Thr Val Leu Val Gln Val
500 505 510

Lys Tyr Glu Gly Thr Asp Ala Pro Cys Lys Ile Pro Phe Ser Thr
515 520 525

Gln Asp Glu Lys Gly Val Thr Gln Asn Gly Arg Leu Ile Thr Ala
530 535 540

Asn Pro Ile Val Thr Asp Lys Glu Lys Pro Ile Asn Ile Glu Thr
545 550 555

Glu Pro Pro Phe Gly Glu Ser Tyr Ile Ile Val Gly Ala Gly Glu
560 565 570

Lys Ala Leu Lys Leu Ser Trp Phe Lys Lys Gly Ser Ser Ile Gly
575 580 585

Lys Met Phe Glu Ala Ile Ala Arg Gly Ala Arg Arg Met Ala Ile
590 595 600

Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Ile Gly Gly Val Phe
605 610 615

Thr Ser Val Gly Lys Leu Val His Gln Val Phe Gly Thr Ala Tyr
620 625 630

Gly Val Leu Phe Ser Gly Val Ser Trp Thr Met Lys Ile Gly Ile
635 640 645

Gly Ile Leu Leu Thr Trp Leu Gly Leu Asn Ser Arg Ser Ala Ser
650 655 660

Leu Ser Met Thr Cys Ile Ala Val Gly Met Val Thr Leu Tyr Leu
665 670 675

Gly Val Met Val

<210> 9
<211> 33

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic DNA

<400> 9
tatcgtacga tgagagttgt gtttgtcgtg cta 33

<210> 10
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic DNA

<400> 10
atagcgcgct tagacagcct tcccaactga 30

<210> 11
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic DNA

<400> 11
tatcgtacga tgcaaaagaa aagaggagga aag 33

<210> 12
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic DNA

<400> 12
atagcgcgct taagcgtgca cgttcacgga g 31

<210> 13
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic DNA

<400> 13
tatcgtacga tgaggtccat agctctcacg 30

<210> 14
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic DNA

<400> 14
atagcgcgct cattaggtct tttcatcatg tctc 34

<210> 15
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic DNA

<400> 15
tatcgtacga tgaacaggag gaaaagatcc gtg 33

<210> 16
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic DNA

<400> 16
atagcgcgct taaaccatga ctcctaggta cag 33

Claims

1. A recombinant virus which is a live attenuated or a defective measles virus comprising a polynucleotide coding for a polypeptide which is a West-Nile virus or respectively a Dengue virus antigen which is selected in the group of:

    - the heterodimer glycoproteins PreM-E or
    - a secreted envelope glycoprotein sE or
    - a polypeptide having at least 80% homology, or 85% homology with the polypeptide having the sequence of SEQ ID NO:6 or SEQ ID NO: 5 or SEQ ID NO:8,

    said recombinant virus being capable of expressing said polypeptide and said polypeptide being capable of inducing a protective immune response against a West-Nile virus in an animal or respectively against a Dengue virus in an animal.

2. The recombinant virus according to claim 1, wherein the polynucleotide encodes a polypeptide selected in the group of:

    - the heterodimer glycoprotein PreM-E which comprises the sequence of SEQ ID NO: 6 or SEQ ID NO: 8 or has the sequence of SEQ ID NO: 6 or SEQ ID NO: 8.
    - the secreted envelope protein sE which has the sequence of SEQ ID NO: 5.

3. The recombinant virus according to any one of claims 1 to 2, wherein said polynucleotide comprises the sequence of SEQ ID NO: 2 or is the sequence of SEQ 10 NO: 1 or comprises the sequence of SEQ ID NO:4.

4. The recombinant measles virus according to any one of claims 1 to 3, comprising, in its genome, said polynucleotide.

5. The recombinant measles virus according to any one of claims 1 to 4, which is derived from the Schwarz measles virus strain.

6. A pharmaceutical composition comprising:

a) at least a recombinant measles virus according to any one of claims 1 to 5; and
b) a pharmaceutically acceptable vehicle or carrier.

7. The pharmaceutical composition of claim 6, capable of inducing a protective immunity against a West-Nile virus in an animal or respectively inducing a protective immunity against a Dengue virus, in an animal.

8. Use of a pharmaceutical composition according to claim 7, as an anti-West-Nile virus agent or respectively an anti-Dengue virus agent, or for the preparation of an anti-West-Nile virus vaccine or respectively an anti-Dengue virus vaccine.

9. A host cell comprising a recombinant virus as defined in any one of claims 1 to 5.

10. Method of producing a recombinant virus for the preparation of an anti-West-Nile virus vaccine, the method comprising the steps of:

a) providing a host cell as defined in claim 9 ;
b) placing the host cell from step a) in conditions permitting the replication of a recombinant virus as defined in any one of claims 1 to 5, which is capable of expressing a said polypeptide; and
c) isolating the recombinant virus produced in step b).

11. The host cell according to claim 9, which is the cell line deposited at the C.N.C.M. under accession number I-3018.

12. Use of an effective amount of at least a recombinant measles virus according to any one of claims 1 to 5, for the preparation of a medicament intended for treating and/or preventing a West-Nile virus infection or respectively a Dengue virus infection, in an animal.

Patentansprüche

1. Rekombinantes Virus, das ein lebend attenuiertes oder ein defektes Masern-Virus ist, umfassend ein Polynukleotid, das für ein Polypeptid kodiert, das ein West-Nil-Virus antigen, beziehungsweise ein Dengue-Virusantigen ist, das ausgewählt ist aus der Gruppe aus:

- dem Heterodimer der Glykoproteine PreM-E oder
- einem sezernierten Hüllglykoprotein sE oder
- einem Polypeptid mit mindestens 80% Homologie oder 85% Homologie mit dem Polypeptid mit der Sequenz von SEQ ID NO: 6 oder SEQ ID NO: 5 oder SEQ ID NO: 8,

wobei das rekombinante Virus zum Exprimieren des Polypeptids fähig ist, und das Polypeptid zum Induzieren einer schützenden Immunantwort gegen ein West-Nil-Virus in einem Tier, beziehungsweise gegen ein Dengue-Virus in einem Tier, fähig ist.

2. Rekombinantes Virus nach Anspruch 1, wobei das Polynukleotid ein Polypeptid kodiert, ausgewählt aus der Gruppe aus:

- dem Heterodimer der Glykoproteine PreM-E, das die Sequenz von SEQ ID NO: 6 oder SEQ ID NO: 8 umfasst, oder das die Sequenz von SEQ ID NO: 6 oder SEQ ID NO: 8 aufweist,
- dem sezernierten Hüllglykoprotein sE, das die Sequenz von SEQ ID NO: 5 aufweist.

3. Rekombinantes Virus nach einem beliebigen der Ansprüche 1 bis 2, wobei das Polynukleotid die Sequenz von SEQ ID NO: 2 umfasst, oder die Sequenz von SEQ ID NO: 1 ist, oder die Sequenz von SEQ ID NO: 4 umfasst.

4. Rekombinantes Masern-Virus nach einem beliebigen der Ansprüche 1 bis 3, umfassend in dessen Genom das Polynukleotid.

5. Rekombinantes Masern-Virus nach einem beliebigen der Ansprüche 1 bis 4, das von dem Schwarz-Masern-Virus-

stamm abgeleitet ist.

6. Pharmazeutische Zusammensetzung, umfassend:

(a) mindestens ein rekombinantes Masern-Virus gemäß einem beliebigen der Ansprüche 1 bis 5; und
(b) ein pharmazeutisch verträgliches Vehikel oder einen pharmazeutisch verträglichen Träger.

7. Pharmazeutische Zusammensetzung nach Anspruch 6, die zum Induzieren einer schützenden Immunantwort gegen ein West-Nil-Virus in einem Tier, beziehungsweise zum Induzieren einer schützenden Immunantwort gegen ein Dengue-Virus in einem Tier, fähig ist.

8. Verwendung einer pharmazeutischen Zusammensetzung nach Anspruch 7 als anti-West-Nil-Virus-Mittel, beziehungsweise anti-Dengue-Virus-Mittel, oder für die Herstellung einer anti-West-Nil-Virus-Vakzine, beziehungsweise einer anti-Dengue-Virus-Vakzine.

9. Wirtszelle, umfassend ein wie in einem beliebigen der Ansprüche 1 bis 5 definiertes rekombinantes Virus.

10. Verfahren zum Herstellen eines rekombinanten Virus zur Herstellung einer anti-West-Nil-Virus-Vakzine, wobei das Verfahren die Schritte umfasst:

a) Bereitstellen einer wie in Anspruch 9 definierten Wirtszelle;
b) Platzieren der Wirtszelle aus Schritt a) unter Bedingungen, die die Replikation eines wie in einem beliebigen der Ansprüche 1 bis 5 definierten rekombinanten Virus, das zum Exprimieren des Polypeptids fähig ist, erlauben; und
c) Isolieren des in Schritt b) hergestellten rekombinanten Virus.

11. Wirtszelle nach Anspruch 9, die die bei der C. N. C. M. unter Zugangsnummer I-3018 hinterlegte Zelllinie ist.

12. Verwendung einer wirksamen Menge von mindestens einem rekombinanten Masern-Virus gemäß einem beliebigen der Ansprüche 1 bis 5, zur Herstellung eines Medikamentes, das zum Behandeln und/oder Vorbeugen einer West-Nil-Virus-Infektion, beziehungsweise einer Dengue-Virus-Infektion, in einem Tier beabsichtigt ist.

Revendications

1. Virus recombinant qui est un virus vivant atténué ou un virus défectueux de la rougeole comprenant un polynucléotide codant pour un polypeptide qui est un antigène d'un virus du Nil occidental ou respectivement un antigène du virus de la dengue qui est choisi dans le groupe constitué par :

- les glycoprotéines hétérodimères PreM-E ou
- une glycoprotéine d'enveloppe sécrétée SE ou
- un polypeptide ayant au moins 80% d'homologie, ou 85% d'homologie avec le polypeptide ayant la séquence de SEQ ID NO: 6 ou SEQ ID NO: 5 ou SEQ ID NO: 8,

ledit virus recombinant étant capable d'exprimer ledit polypeptide et ledit polypeptide étant capable d'induire une réponse immunitaire protectrice contre un virus du Nil occidental chez un animal ou respectivement contre un virus de la dengue chez un animal.

2. Virus recombinant selon la revendication 1, dans lequel le polynucléotide code pour un polypeptide choisi dans le groupe constitué par :

- la glycoprotéine hétérodimère PreM-E qui comprend la séquence de SEQ ID NO: 6 ou SEQ ID NO: 8 ou qui a la séquence de SEQ ID NO: 6 ou SEQ ID NO: 8
- la glycoprotéine d'enveloppe sécrétée SE qui a la séquence de SEQ ID NO: 5.

3. Virus recombinant selon l'une quelconque des revendications 1 à 2, dans lequel ledit polynucléotide comprend la séquence de SEQ ID NO: 2 ou est la séquence de SEQ ID NO: 1 ou comprend la séquence de SEQ ID NO: 4.

4. Virus de la rougeole recombinant selon l'une quelconque des revendications 1 à 3, comprenant, dans son génome,

ledit polynucléotide.

5. Virus de la rougeole recombinant selon l'une quelconque des revendications 1 à 4, qui est dérivé de la souche Schwarz du virus de la rougeole.

6. Composition pharmaceutique comprenant :

a) au moins un virus de la rougeole recombinant selon l'une quelconque des revendications 1 à 5 ; et
b) un véhicule ou un support pharmaceutiquement acceptable.

7. Composition pharmaceutique selon la revendication 6, capable d'induire une immunité protectrice contre un virus du Nil occidental chez un animal ou respectivement d'induire une immunité protectrice contre un virus de la dengue, chez un animal.

8. Utilisation d'une composition pharmaceutique selon la revendication 7, en tant qu'agent anti-virus du Nil occidental ou respectivement en tant qu'agent anti-virus de la dengue, ou pour la préparation d'un vaccin anti-virus du Nil occidental ou respectivement un vaccin anti-virus de la dengue.

9. Cellule hôte comprenant un virus recombinant tel que défini selon l'une quelconque des revendications 1 à 5.

10. Procédé de production d'un virus recombinant pour la préparation d'un vaccin anti-virus du Nil occidental, le procédé comprenant les étapes consistant à :

a) fournir une cellule hôte telle que définie selon la revendication 9 ;
b) placer la cellule hôte de l'étape a) dans des conditions permettant la réplication d'un virus recombinant tel que défini selon l'une quelconque des revendications 1 à 5, qui est capable d'exprimer ledit polypeptide ; et
c) isoler le virus recombinant produit à l'étape b).

11. Cellule hôte selon la revendication 9, qui est la lignée cellulaire déposée à la C.N.C.M. sous le numéro d'ordre I-3018.

12. Utilisation d'une quantité efficace d'au moins un virus de la rougeole recombinant selon l'une quelconque des revendications 1 à 5, pour la préparation d'un médicament destiné au traitement et/ou à la prévention d'une infection par le virus du Nil occidental ou respectivement d'une infection par le virus de la dengue, dans un animal.

Figure 1

atgagagttgtgtttgtcgtgctattgcttttggtggccccagcttacagcttcaactgccttggaatgagcaacagagactt
cttggaaggagtgtctggagcaacatgggtggatttggttctcgaaggcgacagctgcgtgactatcatgtctaaggac
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Figure 2

MRVVFVVLLLLVAPAYSFNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKP
TIDVKMMNMEAVNLAEVRSYCYLATVSDLSTKAACPTMGEAHNDKRADPAFVCRQ
GVVDRGWGNGCGLFGKGSIDTCAKFACSTKAIGRTILKENIKYEVAIFVHGPTTVES
HGNYSTQVGATQAGRFSITPAAPSYTLKLGEYGEVTVDCEPRSGIDTNAYYVMTVG
TKTFLVHREWFMDLNLPWSSAGSTVWRNRETLMEFEEPHATKQSVIALGSQEGAL
HQALAGAIPVEFSSNTVKLTSGHLKCRVKMEKLQLKGTTYGVCSKAFKFLGTPADT
GHGTVVLELQYTGTDGPCKVPISSVASLNDLTPVGRLVTVNPFVSVATANAKVLIEL
EPPFGDSYIVVGRGEQQINHHWHKSGSSIGKAFTTTLKGAQRLAALGDTAWDFGSV
GGVFTSVGKAV

Figure 3

atgcaaaagaaaagaggaggaaagaccggaattgcagtcatgattggcctgatcgccagcgtaggagcagttacc
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Figure 4

MQKKRGGKTGIAVMIGLIASVGAVTLSNFQGKVMMTVNATDVTDVITIPTAAGKNLCI
VRAMDVGYMCDDTITYECPVLSAGNDPEDIDCWCTKSAVYVRYGRCTKTRHSRRS
RRSLTVQTHGESTLANKKGAWMDSTKATRYLVKTESWILRNPGYALVAAVIGWML
GSNTMQRVVFVVLLLLVAPAYSFNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIM
SKDKPTIDVKMMNMEAVNLAEVRSYCYLATVSDLSTKAACPTMGEAHNDKRADPA
FVCRQGVVDRGWGNGCGLFGKGSIDTCAKFACSTKAIGRTILKENIKYEVAIFVHGP
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VMTVGTKTFLVHREWFMDLNLPWSSAGSTVWRNRETLMEFEEPHATKQSVIALGS
QEGALHQALAGAIPVEFSSNTVKLTSGHLKCRVKMEKLQLKGTTYGVCSKAFKFLG
TPADTGHGTVVLELQYTGTDGPCKVPISSVASLNDLTPVGRLVTVNPFVSVATANAK
VLIELEPPFGDSYIVVGRGEQQINHHWHKSGSSIGKAFTTTLKGAQRLAALGDTAWD
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Figure 5

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Figure 6

MNRRKRSVTMLLMLLPTVLAFHLTTRGGEPHMIVSKQERGKSLLFKTSAGVNMCTLI
AMDLGELCEDTMTYKCPRITEAEPDDVDCWCNATDTWVTYGTCSQTGEHRRDKR
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DRGWGNGCGLFGKGSLLTCAKFKCVTKLEGKIVQYENLKYSVIVTVHTGDQHQVG
NETTEHGTIATITPQAPTSEIQLTDYGTLTLDCSPRTGLDFNEVVLLTMKEKSWLVHK
QWFLDLPLPWTSGASTSQETWNRQDLLVTFKTAHAKKQEVVVLGSQEGAMHTALT
GATEIQTSGTTTIFAGHLKCRLKMDKLTLKGMSYVMCTGSFKLEKEVAETQHGTVLV
QVKYEGTDAPCKIPFSTQDEKGVTQNGRLITANPIVTDKEKPINIETEPPFGESYIIVG
AGEKALKLSWFKKGSSIGKMFEAIARGARRMAILGDTAWDFGSIGGVFTSVGKLVH
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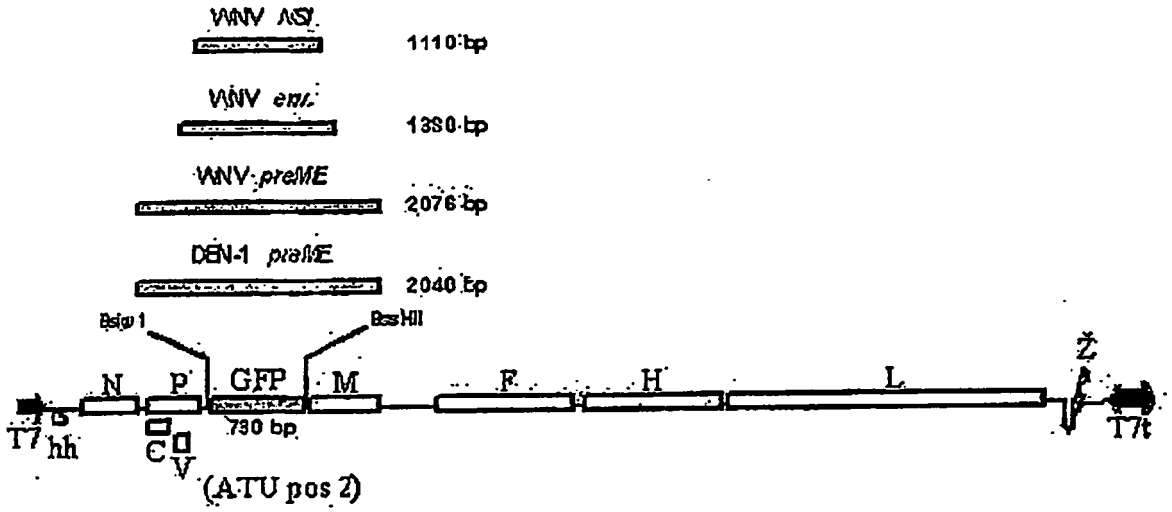
Figure 7



pTM-MVSchw-WNV

Figure 8

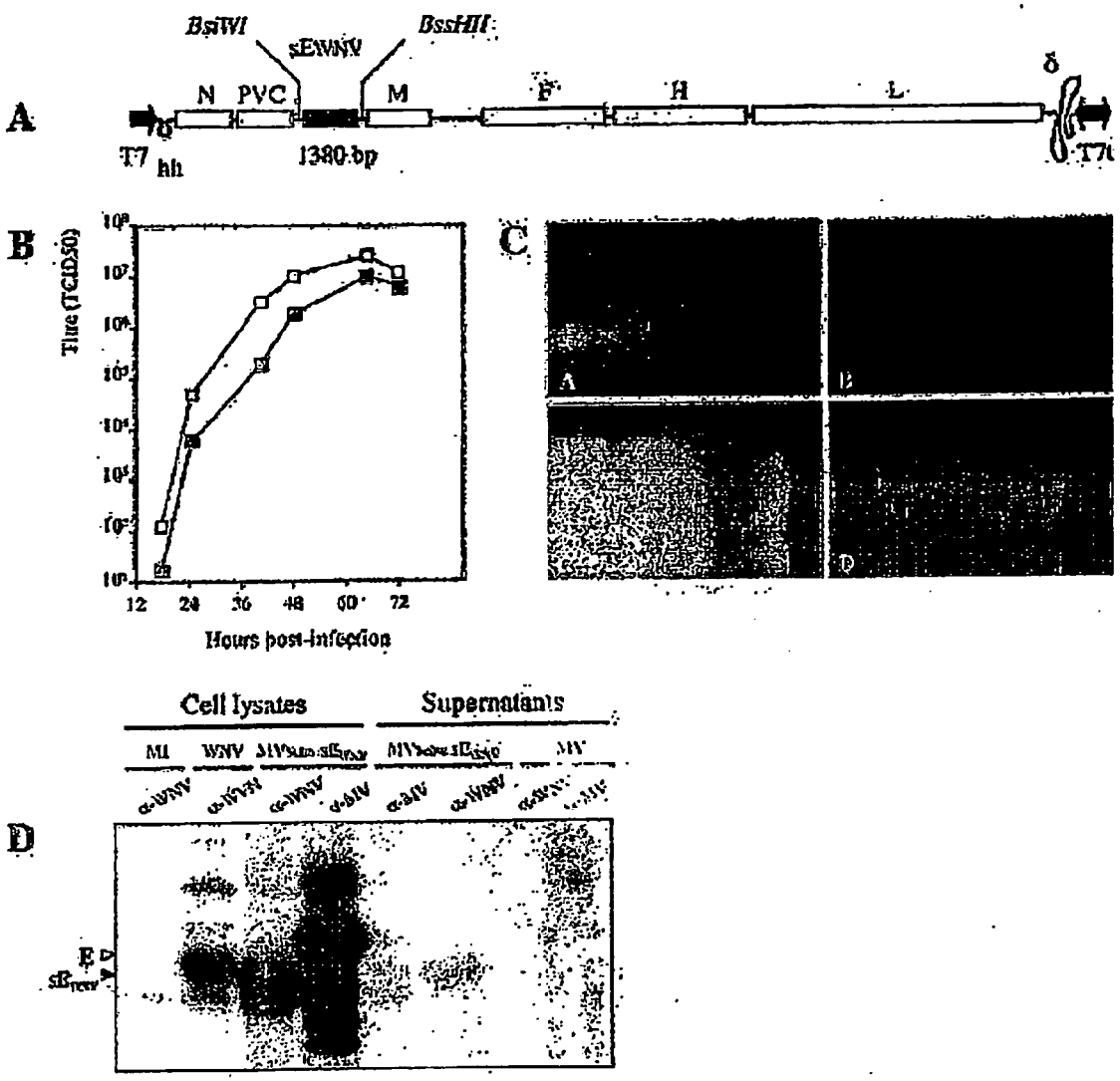
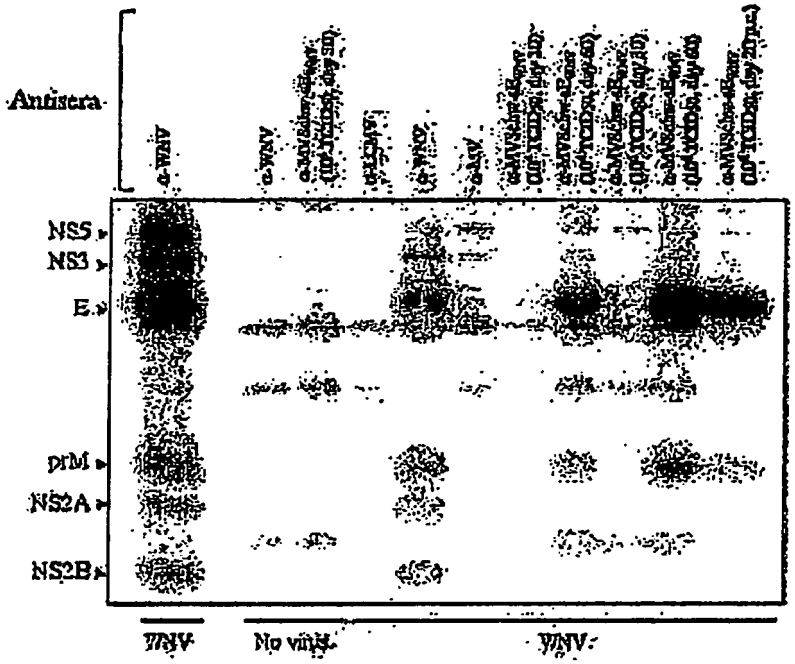

A
BsiWI
sEWNV
BssHII
N
PVC
M
F
H
L
δ
T7
hh
1380 bp
B
Titre (TCID50)
Hours post-infection
C
A
B
D
Cell lysates
Supernatants
M1
WNV
E
sE

Figure 9



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