US 20130011427A1

(19) United States

(12) Patent Application Publication

August et al.

(10) Pub. No.: US 2013/0011427 A1

(43) Pub. Date: Jan. 10, 2013

(54) **FLAVIVIRUS SPECIES-SPECIFIC PEPTIDE TAGS FOR VACCINE AND DIAGNOSTIC USE**

(75) Inventors: **J. Thomas August**, Baltimore, MD (US); **Tin Wee Tan**, Singapore (SG); **Asif Mohammad Khan**, Singapore (SG)

(73) Assignee: **THE JOHNS HOPKINS UNIVERSITY**, Baltimore, MD (US)

(21) Appl. No.: **13/516,501**

(22) PCT Filed: **Dec. 16, 2010**

(86) PCT No.: **PCT/US2010/060777**

§ 371 (c)(1), (2), (4) Date: **Sep. 14, 2012**

Related U.S. Application Data

(60) Provisional application No. 61/287,055, filed on Dec. 16, 2009.

Publication Classification

(51) **Int. Cl.**

A61K 39/12	(2006.01)
C12N 15/40	(2006.01)
A61P 37/04	(2006.01)
C12N 5/10	(2006.01)
G01N 33/53	(2006.01)
C07K 7/06	(2006.01)
C12N 15/63	(2006.01)

(52) **U.S. Cl.** **424/186.1**; 530/328; 536/23.72; 435/320.1; 435/325; 435/69.1; 435/5

(57) **ABSTRACT**

Flaviviruses represent an increasing global public health issue, with no prophylactic and therapeutic formulations currently available for many of them. The combination of factors such as evolutionary change, global warming and wide range of animal hosts suggest the possible occurrence of *Flavivirus* strains with greater distribution and human pathogenicity. There is, thus, a need for greater understanding of viral protein sequences that function in the human immune responses. The evolutionary diversity of the reported sequences of major flaviviruses, such as dengue virus, yellow fever virus, Japanese encephalitis virus, and West Nile virus were analyzed with a combination of experimental and bioinformatics methodologies. The analysis of all reported sequences revealed that these species-specific peptide tags are highly conserved and are potential T-cell epitopes due to correspondence to known or predicted epitopes. These peptide tags have direct relevance to the development of new-generation vaccines and diagnostic applications.

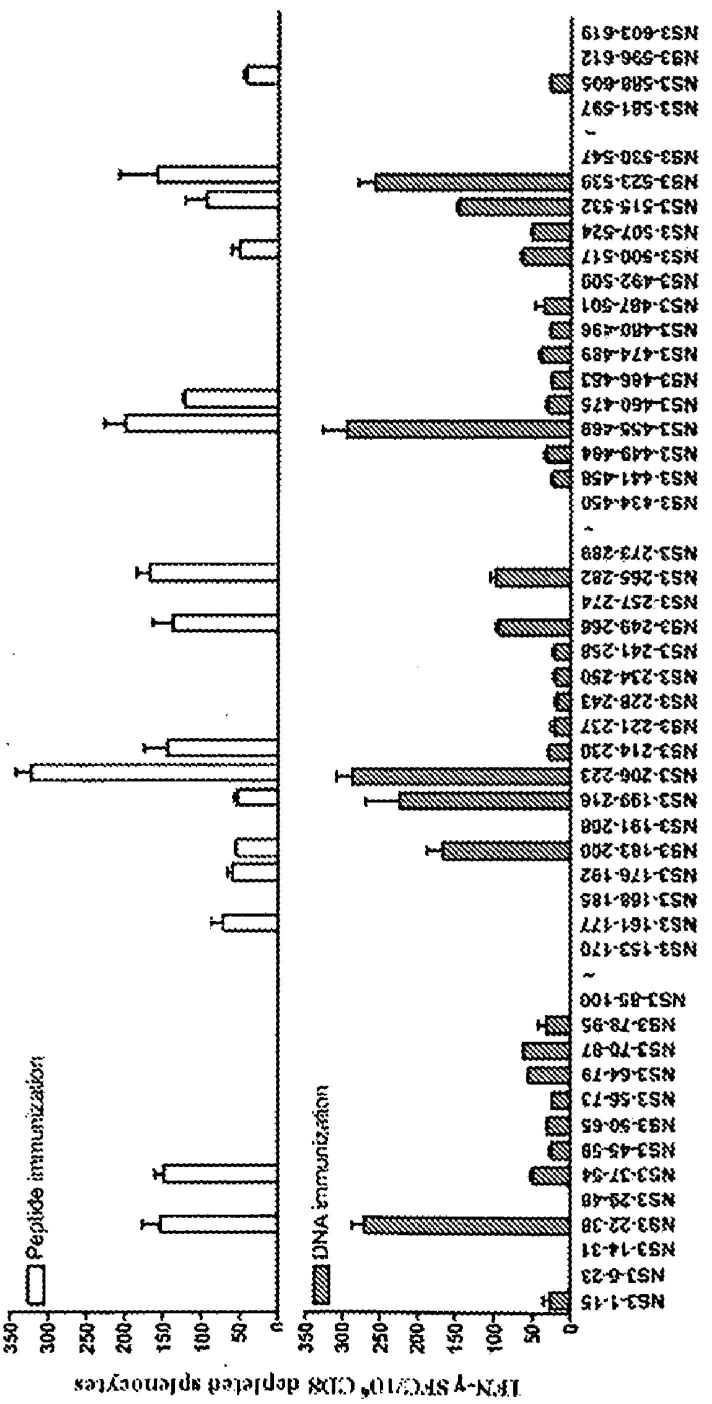

Fig. 1. A comparison of peptide and DNA immunizations.

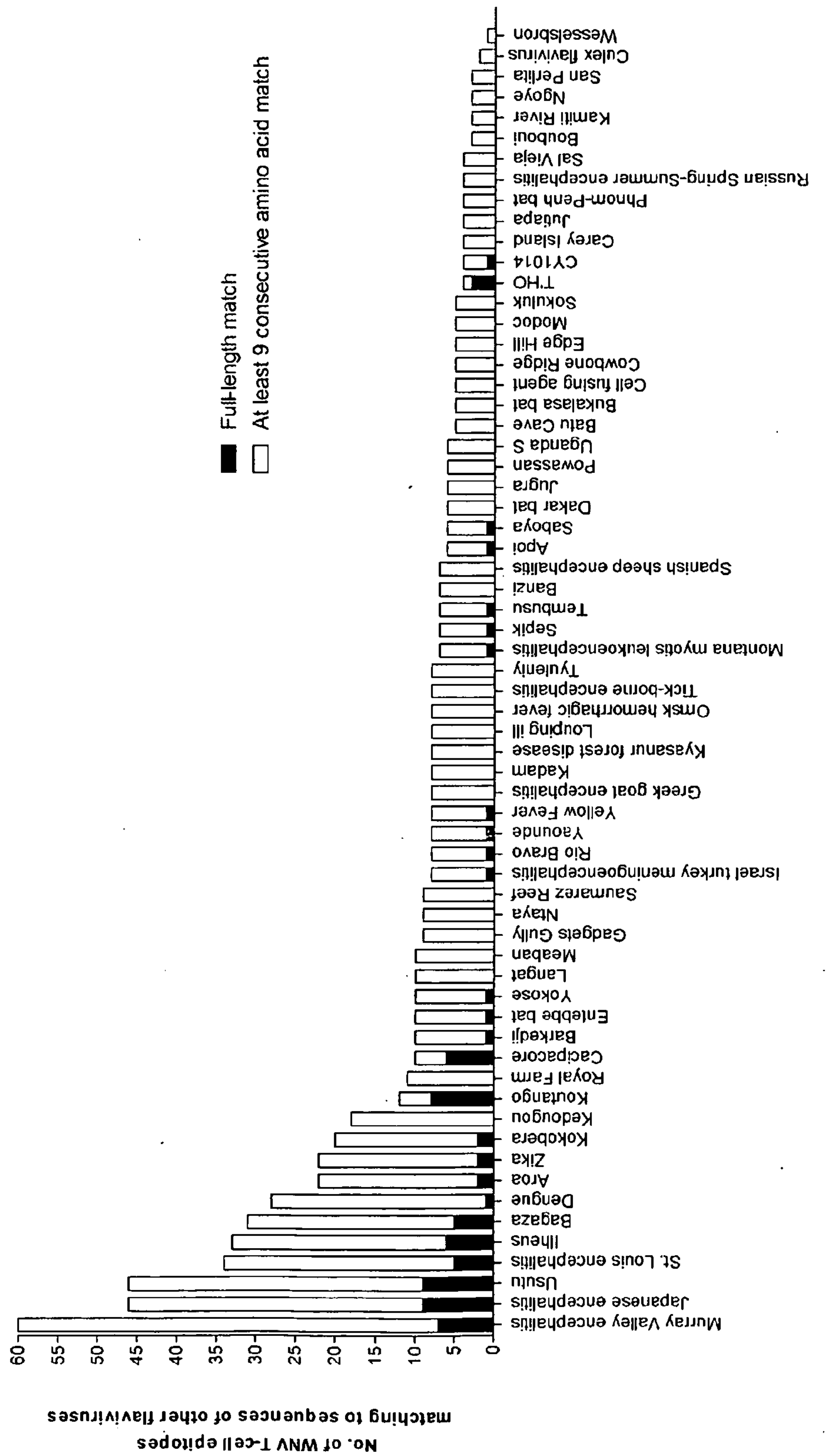

Fig. 2. The number of WNV T-cell epitope peptides conserved in other flaviviruses.

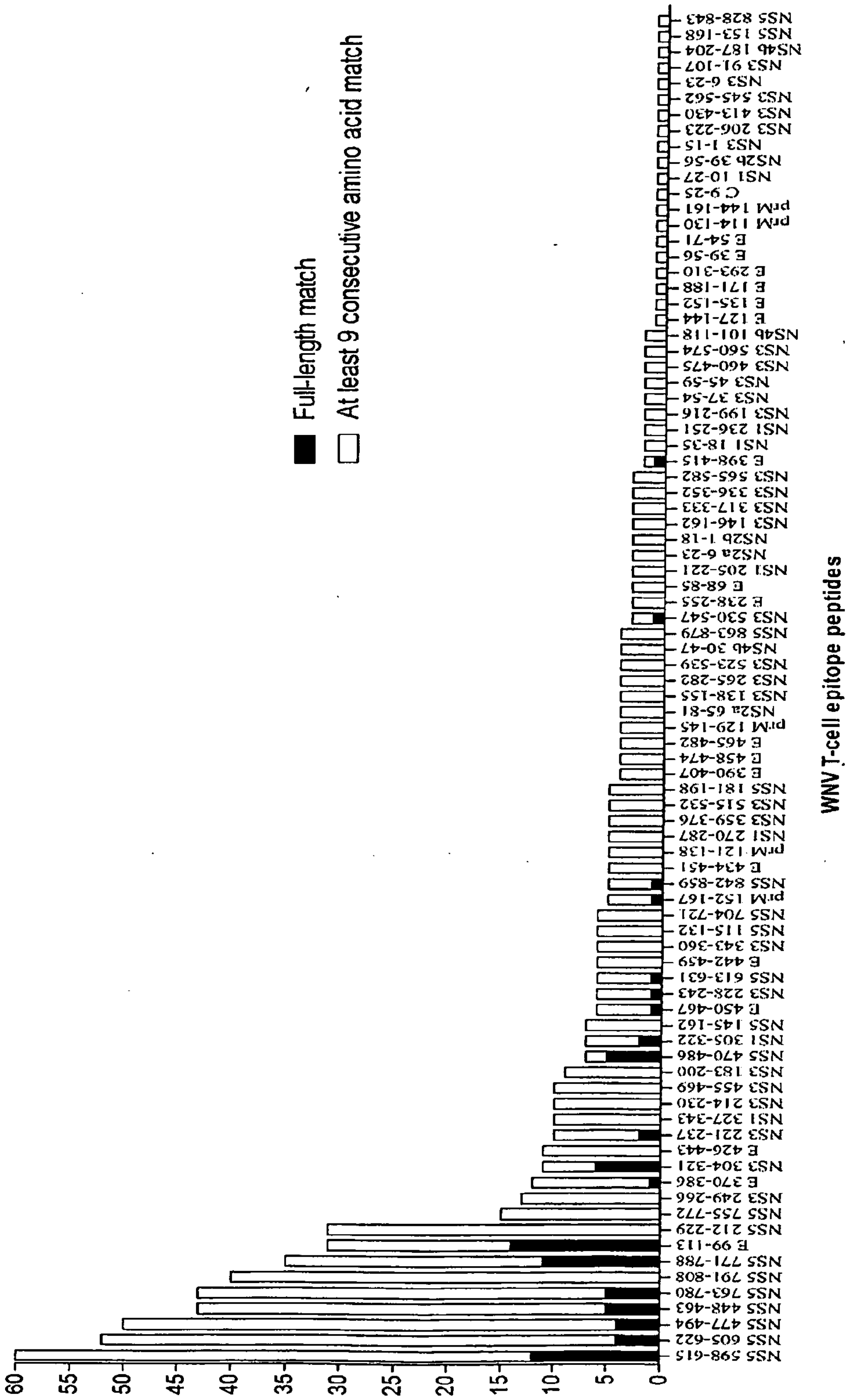

Fig. 3. The number of flaviviruses shared by the individual WNV T-cell epitope peptides.

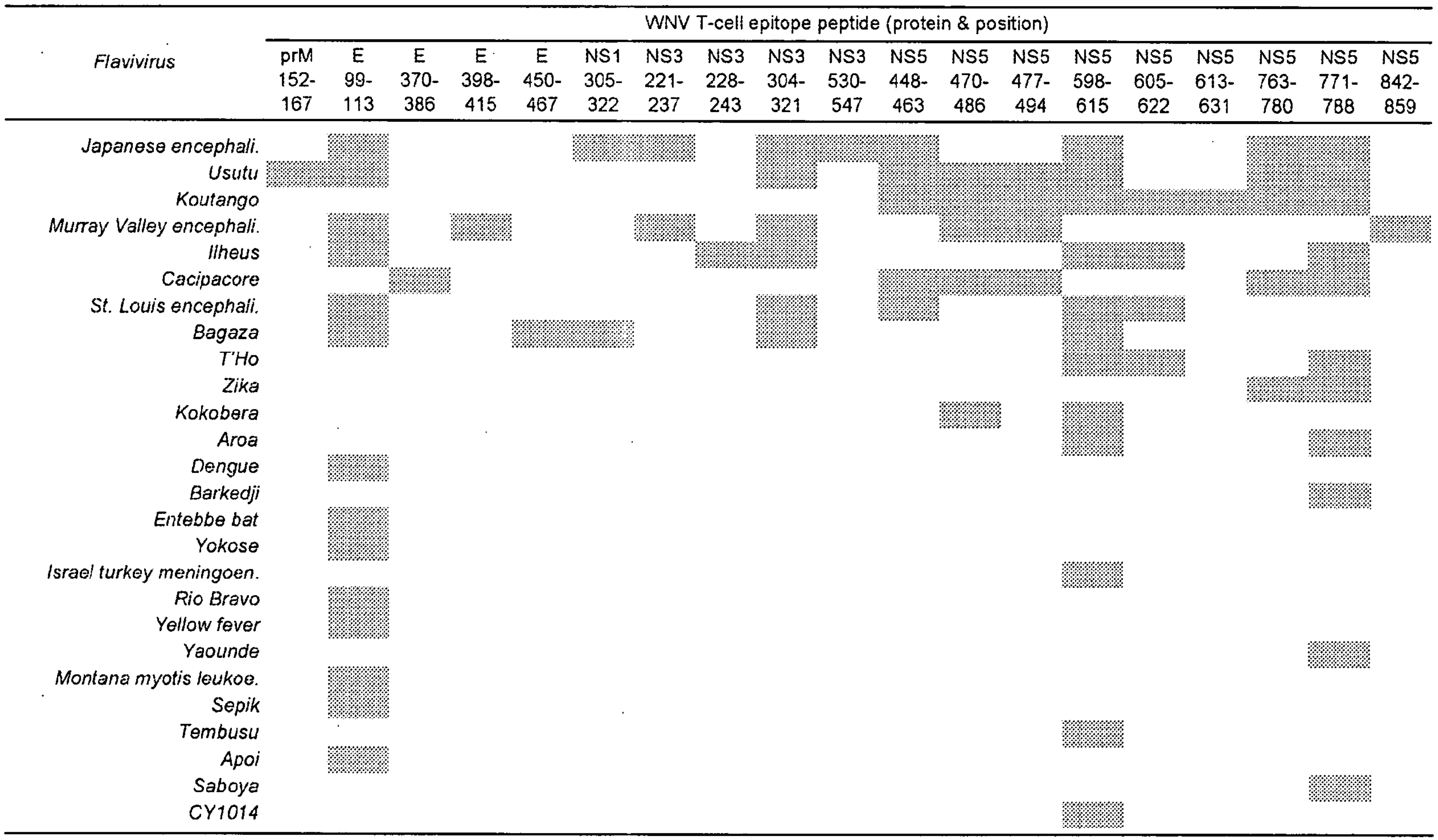

Fig. 4. The distribution of WNV epitope peptides with full-length occurrence in other flaviviruses.

FLAVIVIRUS SPECIES-SPECIFIC PEPTIDE TAGS FOR VACCINE AND DIAGNOSTIC USE

[0001] This invention was made using funding from the U.S. government. Consequently, the U.S. government retains certain rights according to the terms of grant nos. R37A1041908 and U19 A1-056541.

TECHNICAL FIELD OF THE INVENTION

[0002] This invention is related to the area of flaviviruses. In particular, it relates to *Flavivirus* species specific sequences for vaccines, constituents of vaccines, diagnostic, prophylactic, and therapeutic applications.

BACKGROUND OF THE INVENTION

[0003] Flaviviruses, such as West Nile virus (WNV), dengue virus (DENY), Japanese encephalitis virus (JEV), and yellow fever virus (YFV), among others, are arthropod-borne RNA virus pathogens of the genus *Flavivirus* that have high sequence and structural homology (Kuno et al., 1998). The genome of these viruses is a positive-sense, single strand RNA, of approximately 11,000 to 12,000 nucleotides, encoding a polyprotein of approximately 3,430 amino acids that is cleaved to produce three structural proteins, capsid (C), premembrane (prM), membrane (M), and envelope (E), and seven non-structural (NS) proteins, NS1, 2a, 2b, 3, 4a, 4b and 5, with similar structural organization. They have become increasingly important human pathogens. For example, following the introduction of WNV in New York in 1999, the virus has become established throughout the United States as a new genotype (WN02) with multiple genetic and phenotypic changes and more efficient mosquito transmission (Davis et al., 2007; Moudy et al., 2007). Additionally, with global warming, the already widespread dengue viruses have the potential of even greater worldwide distribution. The combined problems of evolutionary change, increasing global distribution, wide range of animal as well as human hosts, and possible occurrence of strains with greater human pathogenicity, call for concerted studies with the goal of developing an effective means to combat future as well as current strains.

[0004] As many RNA viruses are pathogens in humans, there is need for increased understanding of viral protein sequences that function in the human cellular immune responses to these viruses. Several reports have described roles of $CD8^+$ cytolytic T lymphocytes (CTL) and of $CD4^+$ helper T lymphocytes (HTL) in the immune response to a variety of viral infections in animal model systems (BenMohamed et al., 2000; Blaney et al., 1998; Brien et al., 2007; Castrucci et al., 1994; Del Val et al., 1991; La Posta et al., 1993; Lieberman et al., 2007; Oukka et al., 1996; Purtha et al., 2007; Stemmer et al., 1999; Tsuji et al., 1998). However, knowledge of the identities and properties of both CTL and HTL immunogenic peptide sequences of pathogens is limited because of the great diversity in the recognition of the antigen peptides by the host immune system and the thousands of human leukocyte antigen molecules (HLA; human MHC) (Robinson et al., 2006); approximately 3500 reported as of June 2009 (www.ebi.ac.uk/imgt/hla/). There is also the complexity of the genetic structure of single stranded RNA viruses that are among the most variable and adaptable of subcellular parasites resulting from high frequency of point mutations during RNA replication. The genetic change, short generation times, and large virus populations result in rapid evolution dependent upon virus fitness to the vector and host. In almost all cases, the specific genetic changes responsible for viral adaptation are not known because of the stochastic nature of mutagenesis and viral fitness and the complexities of biological responses of the host. Of the great variety of T cell antigenic determinants on WNV proteins, there are only a few for which the structure is known (McMurtrey et al., 2008; Parsons et al., 2008; Wang et al., 2006). However, the advances in pathogen genome sequence data, the development of HLA transgenic mice as a model system, and large-scale synthesis of pathogen peptides has now made possible the systematic analysis of viral proteomes for protein sequences that function as T cell epitopes.

[0005] HLA Transgenic (Tg) mice are widely recognized as a leading model system for analysis of HLA-restricted T cell responses to human pathogens and disease states (Cheuk et al., 2002; Hu et al., 2005; Loirat et al., 2000; Pajot et al., 2004; Pajot et al., 2006; Pascolo, 2005; Richards et al., 2007; Sonderstrup et al., 1999; Taneja and David, 1999).

[0006] There is a continuing need in the art to identify and test *Flavivirus* vaccines to reduce the incidence and/or severity of *Flavivirus* infections and/or epidemics. The selection of evolutionarily conserved protein sequences has widely been considered important to vaccine design in order to limit the selective loss of immunity resulting from mutation and protein modification. However, sequences conserved in the evolution of viruses can be present in many different forms in viruses of related species. It is clear that exposure to multiple flaviviruses by infection or immunization will risk immune responses to a large number of cross-reactive T-cell epitopes that may, as altered peptide ligands (APL), significantly affect the immune responses to the pathogens. Memory T cells selectively engaged by a variant epitope sequence may exhibit an impaired immune response, depending on the positions and types of amino acid substitutions surrounding or within T cell epitopes and the effect of these changes on the affinity of the interaction (Ferrante and Gorski, 2007). The possible effect in humans of APL inhibition or modification of human T cell immune responses has been widely recognized (Sloan-Lancaster and Allen, 1996), particularly in relation to secondary infection and the marked cross-reactivity of memory T cells induced by primary infection followed by re-infection by a second of the four dengue serotypes (Mongkolsapaya et al., 2003; Mongkolsapaya et al., 2006; Screaton and Mongkolsapaya, 2006). The consequences of this may be relevant to the occurrence of dengue hemorrhagic fever (DHF), the more serious manifestation of the dengue virus infection (Rothman, 2004). Thus, we propose that the selection of conserved sequences that are also virus specific should have precedence in vaccine design.

SUMMARY OF THE INVENTION

[0007] According to one aspect of the invention a polypeptide is provided. The polypeptide comprises one or more discontinuous segments of one or more proteins of a *Flavivirus*. The segments comprise at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206.

[0008] Another aspect of the invention is a polynucleotide which encodes a polypeptide. The polypeptide comprises one or more discontinuous segments of one or more proteins of a *Flavivirus*. The segments comprise at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206.

[0009] Yet another aspect of the invention is a nucleic acid vector that comprises the polynucleotide. The polynucleotide encodes the polypeptide. The polypeptide comprises one or more discontinuous segments of one or more proteins of a *Flavivirus*. The segments comprise at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206.

[0010] Still another aspect of the invention is a host cell. The host cell comprises a nucleic acid vector that comprises the polynucleotide. The polynucleotide encodes the polypeptide. The polypeptide comprises one or more discontinuous segments of one or more proteins of a *Flavivirus*. The segments comprise at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206.

[0011] According to another aspect of the invention a method is provided for producing a polypeptide. A host cell is cultured. The host cell comprises a nucleic acid vector that comprises the polynucleotide. The polynucleotide encodes the polypeptide. The polypeptide comprises one or more discontinuous segments of one or more proteins of a *Flavivirus*. The segments comprise at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206. The culturing is under conditions in which the host cell expresses the polypeptide.

[0012] Another aspect of the invention is a method of producing a cellular vaccine. Antigen presenting cells are transfected with a nucleic acid vector that comprises the polynucleotide. The polynucleotide encodes the polypeptide. The polypeptide comprises one or more discontinuous segments of one or more proteins of a *Flavivirus*. The segments comprise at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206. The antigen presenting cells thereby express the polypeptide.

[0013] An additional aspect of the invention is a method of making a vaccine. A polypeptide and an immune adjuvant are mixed together. The polypeptide comprises one or more discontinuous segments of one or more proteins of a *Flavivirus*. The segments comprise at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206.

[0014] A further aspect of the invention is a vaccine composition. The vaccine composition comprises a polypeptide or a polynucleotide encoding the polypeptide. The polypeptide comprises one or more discontinuous segments of one or more proteins of a *Flavivirus*. The segments comprise at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206.

[0015] A further aspect of the invention is a method of immunizing a human or other animal subject. A polypeptide or a nucleic acid vector or a host cell is administered to the human or other animal subject in an amount effective to elicit *Flavivirus*-specific T cell activation. The polypeptide comprises one or more discontinuous segments of one or more proteins of a *Flavivirus*. The segments each comprise at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206. The nucleic acid vector comprises a polynucleotide that encodes the polypeptide. The host cell comprises the nucleic acid vector.

[0016] Another aspect of the invention is a method of identifying a *Flavivirus*. A polynucleotide encoding a polypeptide comprising one or more discontinuous segments of one or more proteins of a *Flavivirus* or its complement is hybridized to the genome of a *Flavivirus*. The segments comprise at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206. Hybridization of the genome to the polynucleotide indicates a species of the *Flavivirus*.

[0017] Yet another aspect of the invention is a method of identifying a *Flavivirus*. Proteins from a virus-infected cell are contacted with an antibody which specifically binds to a polypeptide comprising one or more discontinuous segments of one or more proteins of a *Flavivirus*. The segments comprise at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206. Specific binding to the proteins indicates a species of *Flavivirus*.

[0018] Still another aspect of the invention is a method of identifying a *Flavivirus*. A polypeptide comprising one or more discontinuous segments of one or more proteins of a *Flavivirus* is contacted with a blood sample from a patient. Binding of the polypeptide to an antibody in the blood sample or T cells in the blood sample indicates a species of *Flavivirus*.

[0019] These and other embodiments which will be apparent to those of skill in the art upon reading the specification provide the art with new diagnostic and prophylactic reagents.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIG. **1** shows a comparison of peptide and DNA immunizations.

[0021] FIG. **2** shows the number of WNV T-cell epitope peptides conserved in other flaviviruses.

[0022] FIG. **3** shows the number of flaviviruses shared by the individual WNV T-cell epitope peptides.

[0023] FIG. **4** shows the distribution of WNV epitope peptides with complete full-length occurrence in other flaviviruses.

[0024] Table 1 provides HLA-restricted T-cell epitope peptides of the WNV proteome.

[0025] Table 2 provides West Nile Virus HLA-restricted T-cell epitope peptides, class I and II. SEQ ID NOs: 211-347, in the order as shown.

[0026] Table 3A and 3B provide the apparent functional avidity of WNV T-cell epitope peptides in ELISpot assays of splenocytes from immunized HLA-transgenic mice.

[0027] Table 4 provides highly conserved WNV T-cell epitope peptides, entropy 0.1 or lower.

[0028] Table 5 provides WNV T-cell epitope peptides with high variants incidence.

[0029] Table 6 provides an example of a non-zero entropy WNV epitope peptide site. It commonly includes multiple sequences variant to the epitope, with one or more different amino acid mutations, each of which represented in a small fraction, less than 10%, of the reported sequences.

[0030] Table 7 provides WNV-specific epitope peptides.

[0031] Table 8 provides WNV T-cell epitope peptides with full-length occurrence in other flaviviruses.

[0032] Table 9 provides the distribution of cross-reactive WNV T-cell epitopes in major flaviviruses.

[0033] Table 10 provides variants of highly shared WNV epitope peptides and their incidence in other selected flaviviruses. WNV variant sequences representing less than about 10% of the corresponding database sequences were omitted.

[0034] Table 11 provides WNV HLA-restricted T-cell epitope peptides incidence and their variant incidence distribution. Entropy describing the diversity at the epitope peptide sites is also indicated.

[0035] Table 12 provides list of highly conserved and specific sequences of *Flavivirus* species West Nile virus (WNV), dengue virus (DENV), yellow fever virus (YFV) and Japanese encephalitis virus (JEV). SEQ ID NOs: 1-206, in the order as shown.

DETAILED DESCRIPTION OF THE INVENTION

[0036] The inventors have identified and characterized discontinuous peptide segments from the proteomes of a number of flaviviruses. These are sequences of nine or more contiguous amino acids (aa) are highly conserved in all reported populations of the respective virus species, and are specific to the species, with no matching identity of at least nine aa in any other *Flavivirus*. These sequences are potential HLA-restricted epitope peptides, with many of them shown to be immunogenic in humanized HLA transgenic mice (see, e.g., Table 2 and 7) or predicted to contain T-cell epitope determinants. The identified sequences (in their nucleotide or protein form) have applications to diagnosis of virus infection and the development of new-generation vaccines. Such vaccines may be used either prophylactically or therapeutically, i.e., administered to a person who has not been infected yet or to a person who is already infected.

[0037] Discontinuous segments of the *Flavivirus* may be strung together to form a concatamer, if desired. They may be separated by spacer residues, optionally. Discontinuous segments are those that are not adjacent in the naturally occurring virus isolates. Segments are typically at least 9 amino acid residues and up to about 15, 16, 17, 18, 19, 20, 25, or 30 residues of contiguous amino acid residues from the virus proteome. Single segments may also be used. Because the segments are less than the whole, naturally occurring proteins, and/or because the segments are adjacent to other segments to which they are not adjacent in the proteome, the polypeptides and nucleic acids described here are non-naturally occurring.

[0038] Linkers or spacers with natural or non-naturally occurring amino acid residues may be used optionally. Particular properties may be imparted by the linkers. They may provide a particular structure or property, for example a particular kink or a particular cleavable site. Design is within the skill of the art.

[0039] Polynucleotides which encode the polypeptides may be designed and made by techniques well known in the art. The natural nucleotide sequences used by flaviviruses may be used. Alternatively non-natural nucleotide sequences may be used, including in one embodiment, human codon-optimized sequences. Design of human codon-optimized sequences is well within the skill of the ordinary artisan. Data regarding the most frequently used codons in the human genome are readily available. Optimization may be applied partially or completely.

[0040] The polynucleotides which encode the polypeptides can be replicated and/or expressed in vectors, such as DNA virus vectors, RNA virus vectors, and plasmid vectors. Preferably these will contain promoters for expressing the polypeptides in human or other mammalian or other animal cells. An example of a suitable promoter is the cytomegalovirus (CMV) promoter. Promoters may be inducible or repressible. They may be active in a tissue specific manner. They may be constitutive. They may express at high or low levels, as desired in a particular application. The vectors may be propagated in host cells for expression and collection of chimeric protein. Suitable vectors will depend on the host cells selected. In one embodiment host cells are grown in culture and the polypeptide is harvested from the cells or from the culture medium.

[0041] Suitable purification techniques can be applied to the chimeric protein as are known in the art. In another embodiment one transfects antigen presenting cells for ultimate delivery of the transfected cells to a vaccinee of a cellular vaccine which expresses and presents antigen to the vaccine. Suitable antigen presenting cells include dendritic cells, B cells, macrophages, and epithelial cells.

[0042] Polynucleotides of the invention include diagnostic DNA or RNA oligonucleotides, i.e., short sequences of proven specificity to viral species; these are sufficient to uniquely identify the viral species. Polynucleotides include oliogonucleotides such as primers and probes, which may be labeled or not. These may contain all or portions of the coding sequences for an identified conserved and specific polypeptide. Polynucleotides of the invention and/or their complements, may optionally be attached to solid supports as probes to be used diagnostically, for example, through hybridization to viral genomic sequences. Similarly, epitopic polypeptides can be attached to solid supports to be used diagnostically. These can be used to screen for activated T cells or even antibodies. Suitable solid supports include without limitation microarrays, microspheres, and microtiter wells. Antibodies may be used that are directed against the peptides as disclosed. The antibodies may be used to specifically diagnose a species of *Flavivirus*, for example. Polynucleotides may also be used as primers, for example, of length 18-30, 25-50, or 15-75 nucleotides, to amplify the genetic material of a specific *Flavivirus*. Polynucleotide primers and probes may be labeled with a fluorescent or radioactive label, if desired. These polynucleotides can be used to amplify and/or hybridize to a test sample to determine the presence or species identity of a *Flavivirus*. Such polynucleotides will typically be at least 18, 20, 22, 24, 26, 28, 30, 32, 34 bases in length. Any technique, including but not limited to amplification, hybridization, single nucleotide extension, and sequencing, can be used to identify the presence or species identity of a *Flavivirus*.

[0043] Immune adjuvants may be administered with the vaccines of the present invention, whether the vaccines are polypeptides, polynucleotides, nucleic acid vectors, or cellular vaccines. The adjuvants may be mixed with the specific vaccine substance prior to administration or may be delivered separately to the recipient, either before, during, or, after the vaccine substance is delivered. Some immune adjuvants which may be used include CpG oligodeoxynucleotides, GM-CSF, QS-21, MF-59, alum, lecithin, squalene, and Toll-like receptors (TLRs) adaptor molecules. These include the Toll-interleukin-1 receptor domain-containing adaptor-inducing beta interferon (TRIF) or myeloid differentiation factor 88 (MyD88). Vaccines may be produced in any suitable manner, including in cultured cells, in eggs, and synthetically. In addition to adjuvants, booster doses may be provided. Boosters may be the same or a complementary type of vaccine. Boosters may include a conventional live or attenuated flaviviral vaccine. Typically a high titer of antibody and/or T cell activation is desired with a minimum of adverse side effects.

[0044] Any of the conventional or esoteric modes of administration may be used, including oral, mucosal, or nasal. Additionally intramuscular, intravenous, intradermal, or subcutaneous delivery may be used. The administration efficiency may be enhanced by using electroporation. Optimization of the mode of administration for the particular vaccine composition may be desirable. The vaccines can be administered to patients who are infected already or to patients who do not yet have an infection. The vaccines can thus serve as prophylactic or therapeutic agents. One must, however, bear in mind, that no specific level of efficacy is mandated by the words prophylactic or therapeutic. Thus the agents need not be 100% effective to be vaccines. Vaccines in general are used to reduce the incidence in a population, or to reduce the risk in an individual. They are also used to stimulate an immune response to lessen the symptoms and or severity of the disease.

[0045] The above disclosure generally describes the present invention. All references disclosed herein are expressly incorporated by reference. A more complete understanding can be obtained by reference to the following specific examples, which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

Example 1

[0046] We have applied this system to a large-scale analysis of HLA class I and II-restricted T cell epitopes of the WNV proteome by immunization of 6 mice transgenic for HLA proteins, 3 class 1 and 3 class II, with 452 WNV peptides covering the entire WNV proteome. WNV peptide-specific T-cell responses were assayed by IFN-γ ELISpot and the identified T cell epitope sequences were further analyzed for their apparent avidity in the ELISpot assay, conservation and diversity in the recorded WNV protein sequences, and homology to other *Flavivirus* pathogens. The identification and characterization of these HLA-restricted T cell epitope peptides of the WNV proteome will facilitate further analysis of the human immune response to WNV infection, including application of peptide-specific methodologies for diagnosis for virus infection and the development of new-generation vaccines.

HLA Transgenic Mice

[0047] H-2 class II-deficient, HLA-DR2 (Vandenbark et al., 2003), HLA-DR3 (Madsen et al., 1999; Strauss et al., 1994) and -DR4/human CD4 (huCD4) (Cope et al., 1999; Fugger et al., 1994) Tg mice, and H-2 class I-deficient, HLA-A2.1 (HHD monochain) (Pascolo et al., 1997), HLA-A24/huCD8α (Lemonnier et al., unpublished) and HLA-B7 (Rohrlich et al., 2003) Tg mice were used. HLA-DR2 Tg mice express chimeric molecules, with α1 and β1 domains encoded by the HLA-DRA1*0101 and -DRB1*1501 sequences and the other domains encoded by I-Eα and I-Eβ sequences, from the I-E promoters (Vandenbark et al., 2003). HLA-DR3 Tg mice express the full-length HLA-DRA*0101 and -DRB1*0301 sequences (Madsen et al., 1999; Strauss et al., 1994). HLA-DR4/huCD4 Tg mice express the full-length HLA-DRA*0101 and -DRB1*0401 sequences from the I-Ea promoter, and the human CD4 sequence from the murine CD3δ promoter (Cope et al., 1999; Fugger et al., 1994). HLA-DR2 and -DR3 mice have a homologous deletion of the murine H-2 class II region, and HLA-DR4/huCD4 mice are deficient for I-Aβ and I-Eα. HLA-DR2 mice have a predominant C57BL/6 background, and HLA-DR3 and -DR4/huCD4 mice have mixed backgrounds (B6, B10.H2b, and DBA/1J, 129/Sv, C57BL/6, respectively). HLA-A2.1 (HHD) Tg mice express a chimeric monochain containing the HLA-A*0201α1 and α2 domains and the murine H-2D^b α3 domain linked to human β2-microglobulin (huβ2-m), from the HLA-A2.1 promoter, and are deficient for H-2D and murine β2-m (mβ2-m) (Pascolo et al., 1997). HLA-A24/huCD8α mice express the full-length HLA-A*2402, huβ2-m and huCD8α sequences, and are deficient for H-2K, H-2D, and mβ2-m (Lemonnier et al., unpublished). HLA-B7 mice express a chimeric heavy chain with the HLA-B*0702 α1 and α2 domains and the H-2K^d α3 domain, from the HLA-B7 promoter, and are deficient for H-2K and H-2D (Rohrlich et al., 2003). The three HLA class I Tg strains have been backcrossed for 6 to 12 generations on the C57BL/6 genetic background (Lemonnier, Pasteur Institute). Animals were bred and maintained at the Johns Hopkins School of Medicine Research Animal Resources facilities. Specific pathogen-free (SFP) Tg mice were derived through iodine immersion of neonates (<1 day old) and transfer to outbred foster females (Thompson K and Watson J, The Johns Hopkins School of Medicine). All experiments were approved by the Johns Hopkins Animal Care and Use Committee and carried out according to IACUC guidelines.

Synthetic WNV Peptides

[0048] A library of 452 overlapping peptides covering the entire WNV proteome (NY99-flamingo 382-99 strain), each 14 to 19 amino acids in length with an overlap of 10 residues (>80% purity), was obtained as lyophilized powders from the Biodefense and Emerging Infections Research Resources Repository, NIAID, NTH (Manassas, Va.). Each was dissolved in 100% DMSO and constituted to 20% with sterile filtered water. The final concentration of each peptide was 2 μg/μl. Dissolved peptides were stored at −20° C.

Large Scale Peptide Pool Immunization

[0049] The 6 HLA Tg mice were each immunized with the WNV peptides by use of a peptide pool protocol for large scale T cell epitope identification (Roederer and Koup, 2003). The peptides were divided into 4 immunization pools containing 1 μg of each peptide in groups of about 100 peptides each, as follows: pool 1, 88 peptides spanning the PrM/M and E proteins; pool 2, 107 peptides spanning the N, NS1, NS2a, and NS2b proteins; pool 3, 135 peptides spanning NS 3, NS4a; and NS4b proteins; pool 4, 122 peptides spanning the NS5 protein (data not shown). Each pool was mixed with 50 μl zymosan, 10 mg/ml (Sigma-Aldrich Co, St. Louis, Mo.) in PBS as adjuvant and administered subcutaneously at the base of the tail to groups of 9 to 12 mice of each Tg strain (de la Rosa et al., 2005; Goodridge et al., 2007). Initial matrix assays with peptide pools were performed on day 15-19, after one immunization. Two mice were sacrificed and their splenocytes were selectively depleted of CD8 or CD4 T cells for HLA class II and class I Tg strains, respectively (see below), and T cell responses to peptide pools (10 peptides, 1 μg peptide per pool) were assessed by IFN-γ ELISpot assays. On the following day 2 additional mice were sacrificed and the WNV peptide immunogens identified by the deconvolution analyses were individually tested by ELISpot assay. Experimental values reported herein were obtained with peptide concentration of 10 μg/ml, and a minimum of 3 assays in duplicate with different immunized mice. The remaining mice were immunized a second time on day 21 with the original peptide pool without zymosan. The mice were sacrificed on day 35 and splenocyte T cell responses to individual WNV peptides were further assessed by ELISpot assay with peptide concentrations of 10, 1 and 0.1 μg/ml. Positive control dengue virus peptides immunogenic in the relevant Tg mouse were included in each immunization protocol to evaluate the responses of the individual immunized mouse.

DNA Immunization

[0050] The WNV strain NY99-flamingo382-99 NS3 sequence (GenBank Accession No. AF196835) with NheI and KpnI sites was optimized using the Leto 1.0 software, synthesized by Geneart Inc (Toronto, Canada), and inserted into the p43 vector (Kessler et al., 1996). A p43 vector encoding the dengue virus type 2 prM and E antigens (p-DENV-prM-E) (J. Salmon, unpublished) was used as a control. Four 6-8 weeks old female HLA-DR2 Tg mice were immunized subcutaneously at the base of the tail, twice at 3-week intervals, with 50 μg of the endotoxin-free DNA plasmid p-WNV-NS3, or p-DENV-prM-E. The mice were sacrificed on day 42 and the CD4 T cell responses to the WNV NS3 peptides were assessed by IFN-γ ELISpot assays.

IFN-γ ELISpot Assays

[0051] Ex vivo IFN-γ ELISpot assays were performed using mouse IFN-γ ELISpot sets (BD Biosciences, San Jose, Calif.) following manufacturer's recommendations. Briefly, 96-well ELISpot plates were coated with anti-IFN-γ antibody (5 μg/ml) by incubation at 4° C. overnight, and then blocked with RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 μg/ml streptomycin, and 100 U penicillin, for 2 h at room temperature. Freshly isolated splenocytes from HLA class II and HLA class I Tg mice were depleted of CD8 or CD4 T cells, respectively, using magnetic beads according to the manufacturer's protocol (Miltenyi Biotech, Auburn, Calif.). Flow cytometry analysis of the depleted cells indicated this method routinely achieved >95% depletion of the targeted cells. CD8 or CD4-depleted splenocytes (100 μl containing $0.5-1.0\times10^6$ cells/well) were plated together with WNV peptides. The final concentration of each peptide was 10 μg/ml in the peptide matrix pool and individual peptide validation assays, and 10, 1, and 0.1 μg/ml in the titration assays. Each peptide preparation was tested in duplicated wells. Cells plated without peptide (medium alone) served as negative controls, and concanavalin A (2.5 μg/ml; Sigma-Alrich, St. Louis, Mo.) and known HLA-restricted peptides from dengue virus serotype 3 were included as positive controls. The cells were incubated at 37° C., 5% CO_2 for 16 h. The plates were washed, incubated with biotinylated anti-IFN-γ antibody for 2 h at room temperature, followed by HRP-conjugated streptavidin for 1 h at room temperature. Detection was performed with AEC substrate (Calbiochem, San Diego, Calif.) following manufacturer's instructions. IFN-γ spot-forming cells (SFC) were counted using the Immunospot Series 3B Analyzer ELISPOT reader and Immunospot software version 3.0 (Cellular Technologies, Shaker Heights, Ohio). Experimental values were expressed as the mean numbers of SFC/10^6 CD8- or CD4-depleted splenocytes±SD, after subtraction of values from negative controls (background). Positive ELISpot responses were defined as values above 10, and above the background plus 2 SD. Each ELISpot positive response was confirmed by three assays: matrix screening, individually by the validation assay with the individual peptide, and by peptide titration.

WNV Sequence Data Collection and Processing for Bioinformatics Analysis

[0052] Full-length and partial WNV sequences were retrieved from the NCBI Entrez protein database (Berman et al., 2000; Wheeler et al., 2005) through the NCBI Taxonomy Browser application (taxonomy ID 11082) (as of June 2007). The sequences of the individual WNV proteins were extracted from the collected dataset by performing BLAST (Altschul et al., 1990) search against the downloaded dataset by using the individual protein sequences in the annotated WNV record P06935 as queries. Multiple sequence alignments were performed for each protein with the MUSCLE v3.6 program (Edgar, 2004) and were manually corrected for misalignments when necessary.

Entropy analysis of WNV T-cell epitope sequences

[0053] The evolutionary conservation and variability of the identified T-cell epitope regions in the recorded WNV sequences was measured by use of Shannon entropy computations (Khan et al., 2008; Shannon, 1948) in the Antigenic Variability Analyzer (AVANA) software (Miotto et al., 2008). AVANA was also used to study the representation of the individual epitope sequence and its variants in the corresponding protein alignment. At any given position x in the alignment, variant peptides were defined as those that differed by at least one amino acid from the experimentally identified T cell epitope.

T-Cell Epitope Sequence Homologies with Other Viruses

[0054] Homologs of the WNV T-cell epitopes were searched by performing BLAST analyses of all protein sequences deposited at NCBI (as of January 2009). The parameters set was as follow: limit by Entrez query "Root [ORGN] NOT txid11082[Organism:exp] NOT txid 81077 [ORGN]", "automatically adjust parameters for short sequences" option disabled, "low-complexity" filter disabled, maximum number of aligned sequences to be displayed set to "20,000", expect threshold set to "2,000", word size set to "2", matrix set to "PAM30", gap costs set to "Existence: 9, Extension: 1", compositional adjustments set to "no adjustment". Artificial sequence hits were removed by the "NOT txid81077[ORGN]" keyword.

Example 2

Identification of HLA-Restricted T Cell Epitopes of the WNV Proteome

[0055] Immunization of each of the 6 HLA transgenic mice with 4 peptide pools that comprised the 452 WNV peptides of the entire WNV proteome (data not shown), with zymosan as adjuvant, resulted in the identification of a total of 137 T cell epitope peptides, ~30% of the 452 total, as assayed by IFN-γ ELISpot with splenocytes of the immunized mice (summarized in Table 1; complete data in Table 2). Many (43) of the 137 epitope peptides were immunogenic in multiple, 2 or more, transgenic strains, 25 of which elicited both class I and class II responses, resulting in a total of 200 individual HLA-restricted T cell responses of the 6 transgenic strains, 74 class I (40 A2, 24 A24, 10 B7) and 126 class II (50 DR2, 38 DR3, 38 DR4). These allele-specific T-cell responses to the 452 peptide immunogen thus ranged from ~2% B7 to ~11% DR2, with responses of 6% to 9% of the A2, A24, DR3, and DR4 mice. The M, NS3, and E proteins had the highest concentrations of immunogenic peptides; as a group they represented 160 of the 452 (35%) total peptides, but accounted for 117 (58%) of the 200 T-cell responses. The peptides of preM were non-immunogenic and the least immunogenic were peptides of NC, NS1, and NS5, which collectively elicited only 38 (19%) of the T-cell responses to 193 (43%) of the peptides. Many of the epitope peptides of M, E, and NS3 proteins were in a clustered localization (immunological hotspots). All of the M epitope peptides were in a single cluster of 17 HLA-restricted responses, E contained 3 clusters of the protein amino acids 39-85, 119-152, and 426-482, and almost all of the NS3 peptide epitopes were in clusters of amino acids 1-115, 138-282, 304-376, and 455-605. These 8 clustered regions collectively comprise 65, almost 50%, of the 137 epitope peptide sequences.

TABLE 1

HLA-restricted T-cell epitope peptides of the WNV proteome.

WNV protein	Protein size (aa)[a]	Protein peptides analyzed (#)	ELISpot positive peptides # (%)	HLA-restricted T cell activation A2	A24	B7	DR2	DR3	DR4	Total
C	123	15	1 (~7%)	0	0	1	0	0	0	1
prM	167	21	6 (~29%)	1	2	1	6	3	4	17
E	501	67	25 (~37%)	8	6	2	8	12	6	42
NS1	352	46	10 (~22%)	2	1	0	1	3	5	12
NS2a	231	30	10 (~33%)	0	3	0	8	3	3	17
NS2b	131	16	6 (~38%)	1	3	1	2	0	2	9
NS3	619	84	46 (~55%)	26	0	1	16	11	4	58
NS4a	149	18	5 (~28%)	0	3	0	1	1	2	7
NS4b	255	33	9 (~27%)	0	1	0	3	3	5	12
NS5	905	122	19 (~16%)	2	5	4	5	2	7	25
Total	3433	452	137 (~30%)	40	24	10	50	38	38	200
Frequency allele-specific epitopes per total proteome peptides				9%	5%	2%	11%	8%	8%	

[a]Size indicated in number of amino acids with respect to the flamingo strain (NCBI accession no. AAF20092.2).

TABLE 2

West Nile virus HLA-restricted T-cell epitope peptides, class I and II. (SEQ ID NOs: 211-347)

ELISpot positive peptides		T cell activation of HLA transgenic mice (SFC/10^6) IFN-γ ELISpot					
Protein & position[a]	Peptide sequence[b,c]	A2	A24	B7	DR2	DR3	DR4
C 9-25	GKSRAVN[4]M[1]LKRGMPRVL			43 ± 17			
prM 114-130	STKATR[2]Y[1]L[4]V[5]KTESWILR				101 ± 33		
prM 121-138	L[4]VKTES[4]WI[3]LRNPGYALVA				130 ± 23		56 ± 6
prM 129-145	LRN[3]PG[2]YALVAAVIGWML		71 ± 38	31 ± 7	62 ± 11		
prM 136-153	LVAAVI[4]GW[6]M[5,6]LGSNTMQRV				235 ± 9	78 ± 35	327 ± 162
prM 144-161	M[5,6]LGSN[1]T[1]MQRV[1]VFVVLLLL		59 ± 18		84 ± 32	134 ± 35	315 ± 157
prM 152-167	RV[1]VF[4,6]VV[1]LLLLVAPAYS	318 ± 179			269 ± 30	90 ± 47	117 ± 10
E 39-56	P[5]TID[6]VKM[1]M[6]NMEAANLAEV		137 ± 124				
E 54-71	AEVR[1]SY[6]CY[6]LATVSDLSTK						205 ± 56
E 62-77	LATVSDLSTKAACPTM	465 ± 343					47 ± 4
E 68-85	LSTKAACPTMGEAHNDKR	372 ± 242					
E 99-113	RGWGNGCGLFGKGSI					79 ± 7	
E 119-136	FACSTKAIGRTILKENIK		179 ± 136				
E 127-144	GRTILKENI[4,6]KYEVAIFVH				120 ± 1	198 ± 21	
E 135-152	I[4,6]KYEVAIF[6]VHGPTTVESH					86 ± 13	
E 171-188	PAAPSYTLKLGEYGEVTV	279 ± 217	533 ± 333	996 ± 100			
E 195-212	G[1]IDTNAYY[6]VMTVGTKTFL		137 ± 70				53 ± 16
E 210-225	TF[1]LVHREW[5,6]FMDLNLPW	323 ± 175	454 ± 412	519 ± 477			

TABLE 2-continued

West Nile virus HLA-restricted T-cell epitope peptides, class I and II. (SEQ ID NOs: 211-347)

ELISpot positive peptides		T cell activation of HLA transgenic mice (SFC/10^{6}) IFN-γ ELISpot					
Protein & position[a]	Peptide sequence[b,c]	A2	A24	B7	DR2	DR3	DR4
E 238-255	TLMEFEE^{3}PHATKQSVIAL		122 ± 108				
E 293-310	EKLQ^{1}LKGTT^{2}YGVCSKAFK						47 ± 5
E 356-373	VTV^{4}NPF^{6}VS^{1}VATANAKVLI						217 ± 51
E 370-386	KVLIELEPPFGDSYIVV						148 ± 10
E 384-399	IVVGRGEQQINHHWHK					87 ± 16	
E 390-407	EQQINHHWHKSGSSIGKA					50 ± 6	
E 398-415	HKSGSSIGKAFTTTLKGA				168 ± 18	88 ± 1	
E 426-443	WDFGS^{1}VG^{4}GVFTSVGKAVH					83 ± 13	
E 434-451	VFTSVGKAVHQVFGGAFR				116 ± 5	79 ± 19	
E 442-459	VHQVF^{5}G^{4}GAF^{6}RSLFGGMSW	442 ± 313			117 ± 5	101 ± 15	
E 450-467	FRS^{1}L^{4}FGG^{1}MSWITQGLLGA	205 ± 145			261 ± 13	104 ± 21	
E 458-474	SWITQGL^{1}LGALLLWMGI				51 ± 4		
E 465-482	LGAL^{1,4}L^{6}LWMGINARDRSIA	310 ± 186			490 ± 64	330 ± 91	
E 486-501	LA^{1}VGGV^{1,4}L^{6}L^{6}FLSVNVHA	323 ± 173			214 ± 27	244 ± 16	
NS1 1-19	DTGCAI^{5}DISRQELRCGSGV					572 ± 20	
NS1 10-27	RQELRCGSGVFIHNDVEA						63 ± 8
NS1 18-35	GVFIHNDVEAWMDRYKYY					52 ± 8	
NS1 161-178	GLTSTRMFL^{6}KVRESNTTE				134 ± 8		
NS1 205-221	RLNDTW^{4,6}KLERAVLGEVK						61 ± 15
NS1 228-245	THTLWGDGILESDLIIPV	84 ± 47					
NS1 236-251	ILESDL^{6}IIPVTLAGPR	78 ± 42	43 ± 18				
NS1 270-287	EGRVEIDFDYCPGTTVTL						54 ± 6
NS1 305-322	GKL^{5}ITDWCCRSCTLPPLR					557 ± 25	109 ± 22
NS1 327-343	SGCWY^{5}GMEIRPQRHDEK						78 ± 4
NS2a 6-23	IDPFQ^{1}LGLLV^{1}VFLATQEV		437 ± 62		563 ± 41	45 ± 7	234 ± 8
NS2a 45-61	VFGGI^{5}TYTDVLRYVILV		243 ± 196		307 ± 119		74 ± 11
NS2a 52-66	TDV^{4}LRY^{3,6}VILVGAAFA				516 ± 9		
NS2a 65-81	FAESNSGGDVVHLALMA						60 ± 13
NS2a 80-97	MATF^{6}KIQ^{3}PV^{4}F^{1}MVASFLKA				289 ± 83		
NS2a 128-145	EIPDVLNS^{1}LAVAWMILRA				384 ± 125		
NS2a 136-154	LAVAW^{4}MI^{6}LRAI^{1}TFTTTSNV				98 ± 22		
NS2a 160-177	ALLTPGLRC^{1}L^{5}NLDVYRIL	63 ± 18				59 ± 3	
NS2a 168-183	C^{1}LN^{1}LDV^{4}YRILLLMVGI				556 ± 33	231 ± 16	
NS2a 211-229	G^{1}LFN^{3}PMILAAGLIACDPNR				74 ± 23		

TABLE 2-continued

West Nile virus HLA-restricted T-cell epitope peptides, class I and II. (SEQ ID NOs: 211-347)

ELISpot positive peptides		T cell activation of HLA transgenic mice (SFC/10^6) IFN-γ ELISpot					
Protein & position[a]	Peptide sequence[b,c]	A2	A24	B7	DR2	DR3	DR4
NS2b 1-18	GWPATEVM[1]TAVGLMFAIV						104 ± 18
NS2b 39-56	MFAAFVISGKSTDMWIER				47 ± 18		
NS2b 59-75	DISWESDAEITGSSERV				59 ± 12		
NS2b 84-101	NF[1,6]QL[5]MNDPGAPWKIWMLR		35 ± 1				
NS2b 107-124	ISAYTPW[4,6]AILPSVVGFWI		50 ± 11	81 ± 8			163 ± 4
NS2b 115-131	ILPS[1]VVGFWITLQYTKR	108 ± 60	56 ± 2				
NS3 1-15	GGV[5]LWDTPSPKEYKK			45 ± 12			
NS3 6-23	DTPSPKEYKKGDTTTGVY						43 ± 6
NS3 22-38	V[4]YRIMTRGL[1]LGSYQAGA				155 ± 22		
NS3 37-54	GAGV[1]MVEGVFHTLWHTTK				149 ± 13	368 ± 66	
NS3 45-59	VFHTLW[6]HTTKGAALM					63 ± 10	
NS3 50-65	WHTTKGAA[1]LMSGEGRL					191 ± 37	
NS3 70-87	GSVKEDRL[4]CYGGPWKLQH	174 ± 13					
NS3 78-95	CYGGPWKLQHKWNGQDEV	150 ± 17					
NS3 85-100	LQHKWNG[1]QDEVQMIVV	72 ± 9					
NS3 91-107	G[1]QDEV[6]QMIVVEPGKNVK	156 ± 11					
NS3 98-115	IVVEPGKNVKNVQTKPGV	145 ± 25					
NS3 138-155	PIVDKNGDV[4]IGLYGNGVI	159 ± 10					
NS3 146-162	V[4]I[4]GLY[6]GNGVIMPNGSYI	58 ± 38					
NS3 161-177	YISAIVQGERMDEPIPA	56 ± 28			72 ± 15		
NS3 176-192	PAGFEPEM[1]LRKKQITVL				59 ± 8		
NS3 183-200	M[1]LRKKQITVLDLHPGAGK	62 ± 24			55 ± 2		
NS3 199-216	GKTRRIL[3]PQIIKEAINRR				52 ± 6		
NS3 206-223	PQIIKEAIN[4]RRLRTAVLA	108 ± 22			323 ± 21		
NS3 214-230	NRRL[6]R[4]TA[3]VLAPTRVVAA				146 ± 29		
NS3 221-237	VLAPTRVVAAEMAEALR	26 ± 4					
NS3 228-243	VAAEMA[4]EALRGLPIRY						46 ± 9
NS3 241-258	IRY[6]QTSAVPREHNGNEIV	127 ± 18					
NS3 249-266	PREHNGNEIVDVMCHATL	79 ± 27			139 ± 25		46 ± 16
NS3 265-282	TLT[4]HRLMSPHRVPNYNLF	150 ± 30			169 ± 16		
NS3 304-321	KVELGEAAAIFMTATPPG	94 ± 22					
NS3 317-333	AT[3]PPGTSDPFPESNSPI	151 ± 18					
NS3 324-340	DPFPESNSPISDLQTEI	117 ± 20					

TABLE 2-continued

West Nile virus HLA-restricted T-cell epitope peptides, class I and II. (SEQ ID NOs: 211-347)

ELISpot positive peptides		T cell activation of HLA transgenic mice (SFC/10[6]) IFN-γ ELISpot					
Protein & position[a]	Peptide sequence[b,c]	A2	A24	B7	DR2	DR3	DR4
NS3 336-352	LQTEIPDRAWNSGYEWI	167 ± 35					
NS3 343-360	RAWNSGYEWITEYTGKTV	55 ± 21					
NS3 359-376	TVWF[5,6]VPSVKIAGNEIALCL	75 ± 44					
NS3 413-430	EM[5]G[4]ANF[6]KASR[3]VIDSRKSV	130 ± 15					
NS3 455-469	AAQRRGRIGRNPSQV				200 ± 27		
NS3 460-475	GRIGRNPSQVGDEYCY				123 ± 4		
NS3 474-489	CYGGHTNEDDSNFAHW	92 ± 18				83 ± 23	
NS3 480-496	NEDDSNFAHWTEARIML					121 ± 1	
NS3 487-501	AHWTEARIMLDNINM	308 ± 188				431 ± 112	
NS3 492-509	ARIM[6]LDNINM[3]PNGLIAQF					216 ± 42	
NS3 500-517	NMPNGLIAQFYQPEREKV				51 ± 10	145 ± 49	
NS3 515-532	EKVY[1]TMDGEYRLRGEERK	140 ± 24			95 ± 27		
NS3 523-539	EYRLRGEERKNFLELLR	57 ± 11			160 ± 48		
NS3 530-547	ERKNFL[4]ELLR[1]TADLPVWL	127 ± 10					
NS3 545-562	VW[1]LAYKVAAAGVSYHDRR					110 ± 9	
NS3 560-574	DRRWCF[6]DGPRTNTIL					225 ± 10	
NS3 565-582	FDGPRTNT[1]ILEDNNEVEV					59 ± 3	
NS3 581-597	EVITK[5]LGERKILRPRWI						52 ± 11
NS3 588-605	ERKILR[3]PRWIDARVYSDH				42 ± 4		
NS4a 1-17	S[1]QIG[1]LIEVLGKMPEHFM					132 ± 17	
NS4a 15-31	HF[1]MGK[1]TWEA[1]LDTMYVVA				278 ± 26		187 ± 20
NS4a 54-71	I[1]A[1]L[4]IAL[1]LSV[1]M[1,2]TMGVFFLL		70 ± 16				
NS4a 62-79	V[1]M[1,2]TMGVFFLLMQRKGIGK		117 ± 39				
NS4a 86-103	VLGVAT[1]FFCWMAEVPGTK		65 ± 30				63 ± 1
NS4b 30-47	GEF[1,5,6]LLDL[4]R[3]PA[1]TAWSLYAV				89 ± 21		
NS4b 38-55	PA[1]TAWS[1]L[2,4]Y[6]AVTTAVLTPL				57 ± 11	67 ± 13	
NS4b 63-80	DY[1]I[6]N[4]TS[1]L[6]TSINVQASALF					386 ± 60	92 ± 13
NS4b 87-103	PF[1]VDVGVSALLLAAGCW						59 ± 14

TABLE 2-continued

West Nile virus HLA-restricted T-cell epitope peptides, class I and II. (SEQ ID NOs: 211-347)

ELISpot positive peptides		T cell activation of HLA transgenic mice (SFC/10[6]) IFN-γ ELISpot					
Protein & position[a]	Peptide sequence[b,c]	A2	A24	B7	DR2	DR3	DR4
NS4b 101-118	GCWG[1]QV[6]TLTVTVAATLL						134 ± 8
NS4b 187-204	VV[5]NPSVKTVREAGILITA						83 ± 13
NS4b 201-218	LITAAAV[4]TLWENGASSVW					99 ± 26	73 ± 8
NS4b 233-250	GWLS[1]CL[6]SITW[4,6]TLIKNMEK				62 ± 6		
NS4b 241-255	TW[4,6]TLIKNMEKPGLKR		53 ± 32				
NS5 115-132	L[1]V[4]QS[2]YGWNI[4]VTMKSGVDV					79 ± 23	
NS5 145-162	CDIGESSSSAEVEEHRTI			50 ± 22			
NS5 153-168	SAEV[4]EEHRTIRVLEMV	332 ± 52		112 ± 72	250 ± 24		
NS5 181-198	V[6]KV[1]LCPY[1]MPK[1]VIEKMELL				122 ± 40		
NS5 212-229	SRNSTHEMYWVSRASGNV				168 ± 13		
NS5 448-463	ECHTCIYNMMGKREKK	26 ± 7					
NS5 470-486	AKGSRA[1,4]IW[2]F[1,4]MWLGARFL		183 ± 19				
NS5 477-494	W[2]F[1,4]MWLGARFLEFEALGFL		198 ± 60				
NS5 598-615	REDQRGSGQVVTYALNTF				252 ± 41		
NS5 605-622	GQVV[6]TY[1]A[1,6]L[4,6]NTFTNLAVQL				556 ± 27		240 ± 76
NS5 613-631	NTF[1]TNLAVQLVRMMEGEGV						119 ± 21
NS5 704-721	GWYDWQQVPFCSNHFTEL						65 ± 27
NS5 755-772	DTACLAK[1]S[2]Y[6]AQMWLLLYF		132 ± 62				
NS5 763-780	Y[6]AQMWLLL[5]YFHRRDLRLM			36 ± 16			91 ± 32
NS5 771-788	YFH5RRDL4,6RL[1]**M**ANAICSAV						135 ± 47
NS5 791-808	NWVPTGRTTWSIHAGGEW						78 ± 17
NS5 828-843	WMEDKTPVEKWSDVPY			39 ± 14			
NS5 842-859	PYSGKREDIWCGSLIGTR		180 ± 66				
NS5 863-879	TWAENI[4,5,6]QVAINQVRAII		79 ± 11			121 ± 2	33 ± 1

[a]Sequence positions in boldface are those with more than one FILA-restricted T-cell response.
[b]Underlined amino acids represent overlapping sequences of adjacent epitope peptides.
[c]The amino acid residues with the superscript numbers 1 to 6 refer to the first residue of the HLA class I nonamer binders (1: A*0201; 2: A*2402; and 3: B*0702) or the nonamer core of the HLA class II binders (4: DRB1*1501; 5: DRB1*0301; and 6: DRB1*0401) predicted by the NetMHC 3.2 [48] (www.cbs.dtu.dk/services//NetMHC-3.2/) and NetMHCIIPan 1.0 [49] (www.cbs.dtu.dk/services/NetMHClIpan/) Web-server immunionformatic algorithims. Those sequences without a superscript number 1 to 6 did not have a predicted binder.

[0056] As further characterization of the epitope peptides, their avidity in the IFN-γ ELISpot assay, which can be attributed either to the binding reaction of either the HLA or T-cell receptor molecules, was measured by titration of the peptides over a 100-fold range of concentrations (10, 1, and 0.1 μg/ml). The majority of the peptide epitopes, 37 of 74 HLA-restricted class 1 responses and 83 of 126 class II, demonstrated high functional avidity (Table 3). Notably, while 10 μg/ml resulted in the greatest number (200) of responses, only 54 required the high peptide concentration (10 μg) to elicit T-cell activation following 2 immunizations with the peptide pools. A majority of the assays of the combined class I and class II T-cell responses (120 of 200) were positive at 0.1 μg/ml peptide, including all M and E class II T-cell responses and peptides of each protein except for class I NS3 and NS5, and class II NS3 and NS4b responses. Many of the peptides with high T-cell response scores (>200 SFC per 1×106 splenocytes) demonstrated comparable T-cell responses in assays with 1.0 and 0.1 μg/ml peptide.

TABLE 3A AND 3B

The apparent functional avidity of WNV T-cell epitope peptides in ELISpot assays of splenocytes from immunized HLA-transgenic mice.

WNV	T-cell responses (#)[a]															
protein	L	I	H	Total	L	I	H	Total	L	I	H	Total	L	I	H	Total
3A. HLA class I-restricted T-cell responses																
	A2				A24				B7				A2, A24, B7			
C	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1
prM	0	0	1	1	0	0	2	2	1	0	0	1	1	0	3	4
E	2	2	4	8	0	0	6	6	2	0	0	2	4	2	10	16
NS1	1	0	1	2	0	0	1	1	0	0	0	0	1	0	2	3
NS2a	0	0	0	0	0	0	3	3	0	0	0	0	0	0	3	3
NS2b	0	0	1	1	1	0	2	3	0	1	0	1	1	1	3	5
NS3	7	9	10	26	0	0	0	0	1	0	0	1	8	9	10	27
NS4a	0	0	0	0	1	1	1	3	0	0	0	0	1	1	1	3
NS4b	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1
NS5	1	0	1	2	1	1	3	5	2	2	0	4	4	3	4	11
Total	11	11	18	40	4	2	18	24	6	3	1	10	21	16	37	74
3B. HLA class II-restricted T-cell responses																
	DR-2				DR-3				DR-4				DR-2, -3, -4			
C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
prM	0	0	6	6	0	0	3	3	0	0	4	4	0	0	13	13
E	0	0	8	8	0	0	12	12	0	0	6	6	0	0	26	26
NS1	0	0	1	1	0	0	3	3	2	0	3	5	2	0	7	9
NS2a	0	0	8	8	2	1	0	3	1	0	2	3	3	1	10	14
NS2b	0	0	2	2	0	0	0	0	1	0	1	2	1	0	3	4
NS3	7	3	6	16	6	2	3	11	3	0	1	4	16	5	10	31
NS4a	0	0	1	1	1	0	0	1	1	0	1	2	2	0	2	4
NS4b	2	0	1	3	3	0	0	3	1	1	3	5	6	1	4	11
NS5	1	2	2	5	0	0	2	2	2	1	4	7	3	3	8	14
Total	10	5	35	50	12	3	23	38	11	2	25	38	33	10	83	126

[a]Low (L) avidity T-cell determinants defined as those IFN-γ ELISpot positive only at 10 μg/ml; intermediate (I), 10 and 1 μg/ml; and high (H), 10, 1 and 0.1 μg/ml.

Example 3

Correspondence Between Peptide and DNA Plasmid Immunization

[0057] T-cell epitopes elicited by peptide immunization with adjuvant may differ from those elicited by viral infection because of the many differences in antigen delivery and processing, and the mechanisms involved in activation of the cellular immune response system. As an evaluation of the extent of these possible differences, the T-cell responses of DR2 transgenic mice immunized with NS3 peptides were compared to the responses to a DNA plasmid immunogen encoding the NS3 protein. The DNA construct was designed to encode NS3 as a cytoplasmic protein lacking a signal sequence and a transmembrane domain and therefore possibly subject to a processing pathway comparable to that of the NS3 proteolytically released from the viral proteome polyprotein. All but two of the peptide-specific T-cell responses following peptide immunization were also detected after DNA immunization (FIG. **1**). The correspondence between the two immunizations was greatest with the stronger T-cell responses, especially those of >100 SFC/10^6 CD8 depleted splenocytes. The major difference was a greater number of low T-cell responses (<50 SFC/10^6 CD8 depleted splenocytes) with DNA immunization. The data suggest that while processing pathways can be important in epitope activation of T cells, there are common mechanisms for selection of epitope peptides delivered as extracellular peptides or encoded by DNA for cellular synthesis.

Example 4

Evolutionary Conservation and Diversity of the WNV T-Cell Epitope Peptides

[0058] Analysis of the evolutionary conservation and the variability of the T-cell epitope peptides identified in this study was performed to determine the distribution of these sequences in the known sequence dataset of WNV. A total of 2,746 complete and partial WNV protein sequences were extracted from the NCBI Entrez protein database (as of June 2007): C, 264; prM, 417; E, 927; NS1, 164; NS2a, 143; NS2b, 146; NS3, 146; NS4a, 142; NS4b, 141; and NS5, 256. The majority of WNV T-cell epitope peptides were highly conserved. All but 12 had an entropy of less than 1.0 (average 0.48) (Table 11) and 31 peptides with entropies of 0.1 or less, mainly present in the E, NS3, and NS5 proteins were found as the unmodified sequence in 99% or more of all WNV (Table 4). There were only 7 epitope peptides of the E, NS2a, NS3, NS4A, and NS5 proteins with entropies greater than 0.7 that included variant sequences present in more than 10% (11 to 23%) of the recorded WNV protein sequences (Table 5). However, it is noteworthy that each peptide epitope, except for sequences with entropy of 0.0 (completely conserved epitopes), were represented by multiple variant sequences with one or more amino acid mutations in a small fraction, less than 10%, of the reported sequences, and in many cases, less than 1%. For example, envelope peptide from position 62 to 77 aa with an entropy of 0.5, was present in 94.3% of the database sequences, while the remaining 5.7% of the database sequences were represented by 16 different variants, 14 of which were each present in less than 1.0% of the recorded viral sequences (Table 6). The origin of these apparently rare sequences is uncertain. Possibly, they represent an under-sequenced clade that is common in nature but localized to a region where the virus was not widely studied.

TABLE 4

Highly conserved WNV T-cell epitope peptides, entropy 0.1 or lower.

Protein & position	Peptide sequence	Incidence (%)[a]	Entropy
prM 114-130	STKATRYLVKTESWILR	>99	0.1
prM 121-138	LVKTESWILRNPGYALVA	>99	0.1
E 99-113	RGWGNGCGLFGKGSI	>99	0.1
E 135-152	IKYEVAIFVHGPTTVESH	99	0.1
E 293-310	EKLQLKGTTYGVCSKAFK	99	0.1
E 370-386	KVLIELEPPFGDSYIVV	>99	0.0
E 384-399	IVVGRGEQQINHHWHK	>99	0.1
E 390-407	EQQINHHWHKSGSSIGKA	99	0.1
E 450-467	FRSLFGGMSWITQGLLGA	>99	0.1
E 458-474	SWITQGLLGALLLWMGI	>99	0.1
E 465-482	LGALLLWMGINARDRSIA	99	0.1
NS1 305-322	GKLITDWCCRSCTLPPLR	99	0.1
NS2b 1-18	GWPATEVMTAVGLMFAIV	>99	0.1
NS3 45-59	VFHTLWHTTKGAALM	99	0.1
NS3 50-65	WHTTKGAALMSGEGRL	99	0.1
NS3 138-155	PIVDKNGDVIGLYGNGVI	99	0.1
NS3 183-200	MLRKKQITVLDLHPGAGK	99	0.1
NS3 265-282	TLTHRLMSPHRVPNYNLF	100	0.0
NS3 359-376	TVWFVPSVKMGNEIALCL	99	0.1
NS3 487-501	AHWTEARIMLDNINM	>99	0.1
NS3 565-582	FDGPRTNTILEDNNEVEV	99	0.1
NS5 115-132	LVQSYGWNIVTMKSGVDV	>99	0.1
NS5 470-486	AKGSRAIWFMWLGARFL	>99	0.1
NS5 477-494	WFMWLGARFLEFEALGFL	100	0.0
NS5 598-615	REDQRGSGQVVTYALNTF	100	0.0
NS5 605-622	GQVVTYALNTFTNLAVQL	99	0.1
NS5 613-631	NTFTNLAVQLVRMMEGEGV	99	0.1
NS5 704-721	GWYDWQQVPFCSNHFTEL	100	0.0

TABLE 4-continued

Highly conserved WNV T-cell epitope peptides, entropy 0.1 or lower.

Protein & position	Peptide sequence	Incidence (%)[a]	Entropy
NS5 755-772	DTACLAKSYAQMWLLLYF	>99	0.1
NS5 763-780	YAQMWLLLYFHRRDLRLM	>99	0.1
NS5 771-788	YFHRRDLRLMANAICSAV	100	0.0
NS5 842-859	PYSGKREDIWCGSLIGTR	99	0.1

[a]Percentage incidence (rounded off to the nearest whole number) of the epitope peptide sequence in all reported WNV sequences. Those with >99% incidence do not include 100%. SEQ ID NOs for each peptide are identified in Table 2.

TABLE 5

WNV T-cell epitope peptides with hi h vapriants incidence.

Protein & position[a]	Peptide sequence[b]	Incidence (%)[c]	Entropy
E 119-136	**FACSTKAIGRTILKENIK**	81	0.9
	.......T..........	16	
	...T...T.WI.Q.....	2	
	11 variants	<1 each	
NS2a 168-183	**CLNLDVYRILLLMVGI**	78	1.0
	V	18	
	.........V...I..	1	
	4 variants	<1 each	
NS2a 211-229	**GLFNPMILAAGLIACDPNR**	75	1.3
	.............T.....	12	
	.V..........M......	7	
	.F..........V......	3	
	.M.S.LV............	1	
	3 variants	<1 each	
NS3 317-333	**ATPPGTSDPFPESNSPI**	87	0.7
	..............A..	11	
	4 variants	<1 each	
NS3 343-360	**RAWNSGYEWITEYTGKTV**	69	1.3
	.............I....	23	
	....T........V....	6	
	.............V....	1	
	1 variant	<1	
NS4a 54-71	**IALIALLSVMTMGVFFLL**	80	1.1
	....T.............	11	
	..........SL......	4	
	.V........SL......	3	
	4 variants	<1 each	
NS5 863-879	**TWAENIQVAINQVRAII**	71	1.6
	..............S..	12	
	......H.......SV.	9	
	....D.........S..	2	
	......H.......SL.	2	
	10 variants	<1 each	

[a]Epitope peptide sites (7), each with entropy greater than 0.7, that included at least one variant that was present in more than 10% of the recorded WNV protein sequences.
[b]Evolutionary variants of the WNV predominant epitope peptide sequences (in bold face) are indicated with only the variant amino acids. Variant(s) with less than 1% incidence are indicated by the sum of the number of such variants.
[c]Percentage incidence (rounded off to the nearest whole number) of the epitope peptide sequence and its variants in all WNV sequences analyzed. Those with <1% incidence do not include 0%. SEQ ID NOs for each peptide are identified in Table 2.

TABLE 6

An example of a non-zero entropy WNV epitope peptide site. It commonly includes multiple sequences variant to the epitope, with one or more different amino acid mutations, each of which represented in a small fraction, less than 10%, of the reported sequences.

Protein & position	Peptide sequence[a]	Incidence (%)[b]	Entropy
E 62-77	**LATVSDLSTKAACPTM**	94	0.5
	..S......R......	1	
	..............A.	1	
	.....E..........	<1	
	..S..E...R......	<1	
	A.SATEI.SS......	<1	
	A...............	<1	
	..A.............	<1	
	..I.............	<1	
	..S.............	<1	
	......H......A..	<1	
	......H...T..A..	<1	
	........I.......	<1	
	.............A..	<1	
	V	<1	
	.........N......	<1	
	S...............	<1	

[a]Variant of the epitope sequence (in bold face) are shown with the variable amino acids.
[b]Incidence of the epitope peptide sequence in all reported WNV sequence data. Those with <1% incidence do not include 0%.

Example 5

WNV-Specific T Cell Epitope Peptide Sequences

[0059] A notable finding was that, despite the high WNV protein conservation, only 51 of the 137 HLA-restricted WNV epitope peptides of this study were specific for WNV (Table 7), with the remaining 86 shared among a number of other flaviviruses. The concentration of WNV specific epitope sequences was greatest in the NS2a, NS2b, NS4a, and NS4b proteins, with 23 of the 30 total epitope peptides of these proteins were specific to WNV. In contrast, there were only 28 WNV specific peptides of the total of 107 epitope peptides present in the E, NS1 and NS3 proteins. The WNV specificity of the epitope peptides was not a function of the conservation, which ranged from 75 to 99% of the recorded sequences. Notably, none of the epitope peptides of NS5, which collectively were among the most highly conserved sequences, was WNV specific.

TABLE 7

WNV-specific epitope peptides.

Protein & position	Peptide sequence	Incidence (%)[a]	Entropy
prM 136-153	LVAAVIGWMLGSNTMQRV	94	0.4
E 62-77	LATVSDLSTKAACPTM	94	0.5
E 119-136	FACSTKAIGRTILKENIK	81	0.9
E 195-212	GIDTNAYYVMTVGTKTFL	86	0.9
E 210-225	TFLVHREWFMDLNLPW	95	0.3
E 356-373	VTVNPFVSVATANAICVLI	96	0.3

TABLE 7-continued

WNV-specific epitope peptides.

Protein & position	Peptide sequence	Incidence (%)[a]	Entropy
E 384-399	IVVGRGEQQINHHWHK	99	0.1
E 486-501	LAVGGVLLFLSVNVHA	98	0.2
NS1 1-19	DTGCAIDISRQELRCGSGV	90	0.7
NS1 161-178	GLTSTRMFLKVRESNTTE	87	0.9
NS1 228-245	THTLWGDGILESDLIIPV	87	0.8
NS2a 45-61	VFGGITYTDVLRYVILV	96	0.4
NS2a 52-66	TDVLRYVILVGAAFA	97	0.3
NS2a 80-97	MATFKIQPVFMVASFLKA	89	0.7
NS2a 128-145	EIPDVLNSLAVAWMILRA	85	1.0
NS2a 136-154	LAVAWMILRAITFTTTSNV	89	0.7
NS2a 160-177	ALLTPGLRCLNLDVYRIL	87	0.8
NS2a 168-183	CLNLDVYRILLLMVGI	78	1.0
NS2a 211-229	GLFNPMILAAGLIACDPNR	75	1.3
NS2b 59-75	DISWESDAEITGSSERV	85	0.9
NS2b 84-101	NFQLMNDPGAPWKIWMLR	94	0.5
NS2b 107-124	ISAYTPWAILPSVVGFWI	88	0.6
NS2b 115-131	ILPSVVGFWITLQYTKR	89	0.6
NS3 22-38	VYRIMTRGLLGSYQAGA	98	0.2
NS3 50-65	WHTTKGAALMSGEGRL	99	0.1
NS3 70-87	GSVKEDRLCYGGPWKLQH	97	0.3
NS3 78-95	CYGGPWKLQHKWNGQDEV	87	0.8
NS3 85-100	LQHKWNGQDEVQMIVV	88	0.7
NS3 98-115	IVVEPGICNVKNVQTKPGV	97	0.3
NS3 161-177	YISAIVQGERMDEPIPA	86	0.8
NS3 176-192	PAGFEPEMLRKKQITVL	86	0.4
NS3 241-258	IRYQTSAVPREHNGNEIV	81	1.1
NS3 324-340	DPFPESNSPISDLQTEI	86	0.9
NS3 474-489	CYGGHTNEDDSNFAHW	95	0.4
NS3 480-496	NEDDSNFAHWTEARIML	94	0.4
NS3 487-501	AHWTEARIMLDNINM	99	0.1
NS3 492-509	ARIMLDNINMPNGLIAQF	89	0.6
NS3 500-517	NMPNGLIAQFYQPEREKV	90	0.6
NS3 581-597	EVITKLGERKILRPRWI	91	0.6
NS3 588-605	ERKILRPRWIDARVYSDH	90	0.6
NS4a 1-17	SQIGLIEVLGKMPEHFM	87	0.8

TABLE 7-continued

WNV-specific epitope peptides.			
Protein & position	Peptide sequence	Incidence (%)[a]	Entropy
NS4a 15-31	HFMGKTWEALDTMYVVA	97	0.2
NS4a 54-71	IALIALLSVMTMGVFFLL	80	1.1
NS4a 62-79	VMTMGVFFLLMQRKGIGK	92	0.5
NS4a 86-103	VLGVATFFCWMAEVPGTK	84	1.0
NS4b 38-55	PATAWSLYAVTTAVLTPL	97	0.2
NS4b 63-80	DYINTSLTSINVQASALF	98	0.2
NS4b 87-103	PFVDVGVSALLLAAGCW	96	0.3
NS4b 201-218	LITAAAVTLWENGASSVW	91	0.5
NS4b 233-250	GWLSCLSITWTLIKNMEK	76	1.5
NS4b 241-255	TWTLIKNMEKPGLKR	79	1.3

[a]Percentage incidence (round off to the nearest whole number) in all reported WNV sequence data. Those with >99% incidence do not include 100%. SEQ ID NOs for each peptide are identified in Table 2.

Example 6

Flavivirus-Shared WNV T-Cell Epitope Peptide Sequences

[0060] Representation of WNV epitope peptide sequences of 9 or more contiguous amino acids in other proteins was searched by BLAST analyses of all protein sequences deposited at NCBI (as of January 2009). Almost all flaviviruses, and few other viruses such as sindbis, Simian immunodeficiency and Trichoplusia ni SNPV, shared 1 or more such sequences of the 86 non-WNV specific epitope peptides, from a single WNV sequence in 1 *Flavivirus* to the presence of the NS5 598-615 sequence in 62 flaviviruses (FIGS. **2** and **3**). Sequences of other NS5 epitope peptides and E 99-113 were each present in over 30 flaviviruses and particularly in the closely related Murray Valley encephalitis, Japanese encephalitis, and Usutu viruses. While most of the 86 non-WNV specific epitope peptides were only partially identical by sequences of 9 or more amino acids, 19 of the WNV epitope peptides were represented by complete sequence match in 28 other flaviviruses (Table 8). These complete epitope peptides consisted mainly of the E, NS3, and NS5 sequences, and included several of the most highly conserved sequences with entropy from 0.1 to 0.0. Three WNV epitope peptides (3) (E 99-113, NS5 598-615, and NS5 771-788) with entropies of 0.1 to 0.0 and conserved in >99% or all recorded WNV, were extensively represented in 12 to 14 other flaviviruses (FIG. **4**). Japanese encephalitis and Usutu viruses each contained 9; Koutango, 8; Murray Valley, 7; Ilheus and Cacipacore, 6; St Louis and Bagaza, 5; and several others, 3 or fewer. There was no direct correlation between the diversity of the peptide and the number of flaviviruses that shared the sequence, and epitope peptides with greater entropies were also shared with multiple viruses. Because the 19 WNV epitope peptides were completely conserved in other flaviviruses, it is very likely that these shared sequences are corresponding HLA-restricted T cell epitopes of these viruses (FIG. **4**).

TABLE 8

WNV T-cell epitope peptides with full-length occurrence in other flaviviruses.				
Protein & position	Peptide sequence	Shared flaviviruses (#)[a]	Incidence (%)[b]	Entropy
prM 152-167	RVVEVVLLLLVAPAYS	1	95	0.4
E 99-113	RGWGNGCGLFGKGSI	14	>99	0.1
E 370-386	KVLIELEPPFGDSYIVV	1	>99	0.0
E 398-415	HKSGSSIGKAFTTTLKGA	1	97	0.3
E 450-467	FRSLFGGMSWITQGLLGA	1	>99	0.1
NS1 305-322	GKLITDWCCRSCTLPPLR	2	99	0.1
NS3 221-237	VLAPTRVVAAEMAEALR	2	90	0.7
NS3 228-243	VAAEMAEALRGLPIRY	1	90	0.6
NS3 304-321	KVELGEAAAIFMTATPPG	6	95	0.3
NS3 530-547	ERKNFLELLRTADLPVWL	1	92	0.4
NS5 448-463	ECHTCIYNMMGKREKK	5	92	0.5
NS5 470-486	AKGSRAIWFMWLGARFL	5	>99	0.0
NS5 477-494	WFMWLGARFLEFEALGFL	4	100	0.0
NS5 598-615	REDQRGSGQVVTYALNTF	12	100	0.0

TABLE 8-continued

WNV T-cell epitope peptides with full-length occurrence in other flaviviruses.				
Protein & position	Peptide sequence	Shared flaviviruses (#)[a]	Incidence (%)[b]	Entropy
NS5 605-622	GQVVTYALNTFTNLAVQL	4	99	0.1
NS5 613-631	NTFTNLAVQLVRMMEGEGV	1	99	0.1
NS5 763-780	YAQMWLLLYFHRRDLRLM	5	>99	0.1
NS5 771-788	YFHRRDLRLMANAICSAV	11	100	0.0
NS5 842-859	PYSGKREDIWCGSLIGTR	1	98	0.1

[a]Number of shared flaviviruses other than WNV.
[b]Percentage incidence (round off to the nearest whole number) of the epitope peptide sequence in all reported WNV sequence data. Those with >99% incidence do not include 100%. SEQ ID NOs for each peptide are identified in Table 2.

Example 7

Representation of WNV T-Cell Epitope Peptides and their Variants in Major Flaviviruses

[0061] Further analysis of the sequence representation of the WNV epitope peptides was performed with other flaviviruses that had adequate database information. These included the Japanese encephalitis group [JEV, LEV (St. Louis EV)]; the tick-borne encephalitis virus group [TBEV, PV (powassan virus)]; yellow fever virus (YFV); and dengue virus (DV). The representation of many of the epitope peptides ranged from low (~1%) to high (100%) among known sequences of the highly studied flaviviruses (Table 9). In particular, the epitopes from the NS5 protein were observed to be highly represented among many of the major flaviviruses, with identical or mutated sequences highly specific to the individual flaviviruses (Table 10). For example, the epitope peptide $NS5_{212-229}$ is present as the dominant sequence of the recorded WNV sequences (130 of 143) and not in any of the other selected viruses; however, specific mutant variants of this sequence were predominant peptides in LEV (26 or 29) and YFV (19 or 22); and several forms were present in dengue viruses with significant representation. The NS5 448-463 peptide and other WNV NS5 epitope peptides were either unique to WNV or shared with members of the closely related JEV group (LEV and/or JEV), and mutated forms were predominant peptide sequences in members of other less related flaviviruses of the tick-borne encephalitis virus group (TBEV and Powassan virus), yellow fever virus, and dengue. It thus is apparent that multiple forms of many WNV epitope peptide variants, that have only minor representation in the WNV database, are not specific for WNV and are widely present as predominant peptide sequences in other flaviviruses.

TABLE 9

The distribution of cross-reactive WNV T-cell epitope peptides in other major *flaviviruses*.							
		Percentage incidence (%) [b]					
Protein & position	Peptide sequence [a]	LEV	JEV	TBEV	PV	YFV	DENV
prM 114-130	STKATRYLVKTESWILR		0|1				
prM 121-138	LVKTESWILRNPGYALVA	0|100					0|9
prM 129-145	LRNPGYALVAAVIGWML	0|100					
prM 152-167	RVVFVVLLLLVAPAYS		0|97				
E 99-113	**RGWGNGCGLFGKGSI**	100|100	94|97	0|>99		47|93	<1|>99
E 127-144	GRTILKENIKYEVAIFVH	0|98					
E 135-152	IKYEVAIFVHGPTTVESH	0|96					
E 238-255	TLMEFEEPHATKQSVIAL	0|98					
E 293-310	EKLQLKGTTYGVCSKAFK		0|1				
E 370-386	KVLIELEPPFGDSYIVV	0|93	0|99				0|41
E 426-443	WDFGSVGGVFTSVGKAVH				0|50		0|55
E 434-451	VFTSVGKAVHQVFGGAFR	0|99	0|98				0|8
E 442-459	VHQVFGGAFRSLFGGMSW	0|98	0|98				
E 450-467	FRSLFGGMSWITQGLLGA	0|100	0|99				
E 458-474	SWITQGLLGALLLWMGI	0|100	0|3				
E 465-482	LGALLLWMGINARDRSIA	0|100	0|4				
NS1 205-221	RLNDTWKLERAVLGEVK		0|94				
NS1 270-287	EGRVEIDFDYCPGTTVTL	0|97	0|2				
NS1 305-322	**GKLITDWCCRSCTLPPLR**		2|98				0|97
NS1 327-343	SGCWYGMEIRPQRHDEK	0|100	0|98				0|100
NS2a 6-23	IDPFQLGLLVVFLATQEV		0|98				
NS2b 1-18	GWPATEVMTAVGLMFAIV		0|94				
NS3 1-15	GGVLWDTPSPKEYKK						0|36

TABLE 9-continued

The distribution of cross-reactive WNV T-cell epitope peptides in other major *flaviviruses*.

		Percentage incidence (%) [b]					
Protein & position	Peptide sequence [a]	LEV	JEV	TBEV	PV	YFV	DENV
NS3 6-23	DTPSPKEYKKGDTTTGVY		0|98				
NS3 37-54	GAGVMVEGVFHTLWHTTK		0|97				
NS3 45-59	VFHTLWHTTKGAALM		0|97				
NS3 138-155	PIVDKNGDVIGLYGNGVI						0|3
NS3 146-162	VIGLYGNGVIMPNGSYI						0|3
NS3 214-230	NRRLRTAVLAPTRVVAA	0|100	0|100				0|66
NS3 221-237	**<u>VLAPTRVVAAEMAEALR</u>**	0|100	90|100				0|66
NS3 228-243	VAAEMAEALRGLPIRY		0|95				0|41
NS3 249-266	PREHNGNEIVDVMCHATL	0|100	0|100	0|100			
NS3 265-282	TLTHRLMSPHRVPNYNLF		0|100				
NS3 304-321	**<u>KVELGEAAAIFMTATPPG</u>**	67|100	98|100				0|100
NS3 343-360	RAWNSGYEWITEYTGKTV		0|75				0|24
NS3 359-376	TVWFVPSVKMGNEIALCL	0|100	0|95				
NS3 455-469	AAQRRGRIGRNPSQV	0|81				0|100	0|73
NS3 460-475	GRIGRNPSQVGDEYCY	0|81	0|2				
NS3 515-532	EKVYTMDGEYRLRGEERK		0|98				0|72
NS3 523-539	EYRLRGEERKNFLELLR	0|100	0|2				
NS3 530-547	**<u>ERKNFLELLRTADLPVWL</u>**		2|100				
NS3 545-562	VWLAYKVAAAGVSYHDRR						0|20
NS3 560-574	DRRWCFDGPRTNTIL		0|100				
NS3 565-582	FDGPRTNTILEDNNEVEV	0|100					
NS4b 30-47	GEFLLDLRPATAWSLYAV		0|100				
NS4b 101-118	GCWGQVTLTVTVTAATLL		0|7				
NS5 145-162	CDIGESSSSAEVEEHRTI					0|86	
NS5 181-198	VKVLCPYMPKVIEKMELL	0|64	0|100				
NS5 212-229	SRNSTHEMYWVSRASGNV	0|93		0|100	0|100	0|100	0|99
NS5 448-463	**<u>ECHTCIYNMMGKREKK</u>**	96|98	93|98	0|100	0|88	0|100	0|>99
NS5 470-486	AKGSRAIWFMWLGARFL		0|100				
NS5 477-494	WFMWLGARFLEFEALGFL	0|100	0|100	0|100		0|100	0|>99
NS5 598-615	**<u>REDQRGSGQVVTYALNTF</u>**	100|100	98|100	0|100	0|95	0|100	0|3
NS5 605-622	**<u>GQVVTYALNTFTNLAVQL</u>**	100|100	0|100	0|100	0|90	0|100	
NS5 613-631	NTFTNLAVQLVRMMEGEGV	0|100					
NS5 704-721	GWYDWQQVPFCSNHFTEL		0|98				0|61
NS5 755-772	DTACLAKSYAQMWLLLYF	0|97	0|95				0|<1
NS5 763-780	**<u>YAQMWLLLYFHRRDLRLM</u>**		90|100				0|>99
NS5 771-788	**<u>YFHRRDLRLMANAICSAV</u>**	0|97	90|100				0|>99
NS5 791-808	NWVPTGRTTWSIHAGGEW	0|100		0|97	0|100		
NS5 828-843	WMEDKTPVEKWSDVPY						0|34
NS5 842-859	PYSGKREDIWCGSLIGTR	0|100	0|100				
NS5 863-879	TWAENIQVAINQVRAII		0|25				0|34

[a] The WNV epitope peptide sequences that had 9 or more consecutive amino acids shared with any of the six other *flaviviruses*. WNV epitope peptide sequences that have a full-length match to the sequences of any one of the six other *flaviviruses* are shown in boldface and underlined.

[b] Percentage incidence is depicted as "X|Y" where "X" refers to the percentage of the total virus sequences analyzed with full-length match to the WNV epitope peptide, and "Y" refers to the percentage of total virus sequences studied with ≧9 consecutive amino acids match to the WNV epitope. The "X" value is shaded (X)

when there is a full-length match in the respective virus. The *Flavivirus* species abbreviations: LEV, St. Louis *encephalitis* virus; JEV, Japanese *encephalitis* virus; TBEV, Tick-borne *encephalitis* virus; PV, *Powassan* virus; YFV, Yellow fever virus and DENY, Dengue virus. SEQ ID NOs for each peptide are identified in Table 2.

TABLE 10

Variants of highly shared WNV epitope peptides and their incidence in other selected flaviviruses. WNV variant sequences representing less than about 10% of the corresponding database sequences were omitted.

WNV epitope peptide and its variant [a]	Representation [b] of the peptides in the respective flaviviruses (# of sequences analyzed)						
NS5 212-229	WNV (143)	LEV (29)	TBEV (29)	PV (21)	YFV (22)	DENV (1309)	Total
SRNSTHEMYWVSRASGNV	130	0	0	0	0	0	130
............H....I	11	0	0	0	0	0	11
............CGT..I	0	0	0	0	0	471	471
..........I.NGT..I	0	0	0	0	0	290	290
............N.T..I	0	0	0	0	0	244	244
............N....I	0	0	0	0	0	243	243
............G.A..I	0	26	0	0	0	0	26
.........Y..G.RS..	0	0	0	0	19	0	19
NS5 448-463	WNV (182)	LEV (45)	JEV (60)	TBEV (33)	PV (24)	YFV (22)	Total
ECHTCIYNMMGKREKK	167	43	56	0	0	0	266
Q.RH.V..........	0	0	0	0	21	0	21
NS5 477-494	WNV (181)	LEV (48)	JEV (60)	TBEV (33)	YFV (23)	DENV (1372)	Total
WFMWLGARFLEFRALGFL	181	0	0	0	0	0	181
.Y................	0	46	0	0	3	541	590
.Y...............M	0	0	0	0	0	474	474
.Y......Y.........	0	0	0	0	20	344	364
........Y.........	0	0	60	0	0	0	60
.Y....S...........	0	0	0	33	0	0	33
NS5 598-615	WNV (182)	LEV (28)	JEV (59)	TBEV (30)	PV (21)	DENV (1379)	Total
REDQRGSGQVVTYALNTF	182	28	58	0	0	0	268
.K........G..G....	0	0	0	0	0	477	477
NS5 605-622	WNV (182)	LEV (28)	JEV (59)	TBEV (30)	PV (21)	YFV (22)	Total
GQVVTYALNTFTNLAVQL	180	28	0	0	0	0	208
..............S...	2	0	0	0	0	0	2
..........L..IK...	0	0	0	30	0	0	30
.............I....	0	0	58	0	0	0	58
..........I...K...	0	0	0	0	0	21	21
..........I..MK...	0	0	0	0	17	0	17

[a] The epitope sequences are shown in bold face and the mutations in the variant peptides are shown by the respective variant amino acids.
[b] Data was collected from the NCBI Entrez Protein Database (as of January 2009) SEQ ID NOs for each peptide are identified in Table 2.

TABLE 11

WNV HLA-restricted T-cell epitope peptides incidence and their variant incidence distribution. Entropy describing the diversity at the epitope peptide sites is also indicated.

		ELISpot positive peptides		Variant peptides [c]		
Protein & position	Entropy [a]	Peptide sequence	Incidence (%) [b]	>10% (#)	1-10% (#)	<1% (#)
C 9-25	0.7	GKSRAVNMLKRGMPRVL	88	—	1	5
prM 114-130	0.1	STKATRYLVKTESWILR	>99	—	—	3
prM 121-138	0.1	LVKTESWILRNPGYALVA	>99	—	—	2
prM 129-145	0.3	LRNPGYALVAAVIGWML	95	—	1	2
prM 136-153	0.4	LVAAVIGWMLGSNTMQRV	94	—	1	4
prM 144-161	0.6	MLGSNTMQRVVFVVLLLL	92	—	3	4
prM 152-167	0.4	RVVFVVLLLLVAPAYS	95	—	2	3
E 39-56	0.3	PTIDVKMMNMEAANLAEV	96	—	1	9
E 54-71	0.3	AEVRSYCYLATVSDLSTK	96	—	1	11
E 62-77	0.5	LATVSDLSTKAACPTM	94	—	2	14
E 68-85	0.4	LSTKAACPTMGEAHNDKR	95	—	2	9
E 99-113	0.1	RGWGNGCGLFGKGSI	>99	—	—	3
E 119-136	0.9	FACSTKAIGRTILKENIK	81	1(16%)	1	11
E 127-144	0.3	GRTILICENIKYEVAIFVH	97	—	1	7
E 135-152	0.1	IKYEVAIFVHGPTTVESH	99	—	—	8
E 171-188	0.6	PAAPSYTLKLGEYGEVTV	91	—	2	12
E 195-212	0.9	GIDTNAYYVMTVGTKTFL	86	—	2	11
E 210-225	0.3	TFLVHREWFMDLNLPW	95	—	1	7
E 238-255	0.3	TLMEFEEPHATKQSVIAL	97	—	1	8
E 293-310	0.1	EKLQLKGTTYGVCSKAFK	99	—	—	6
E 356-373	0.3	VTVNPFVSVATANAKVLI	96	—	1	8
E 370-386	0	KVLIELEPPFGDSYIVV	>99	—	—	2
E 384-399	0.1	IVVGRGEQQINHHWHK	>99	—	—	4
E 390-407	0.1	EQQINHHWHKSGSSIGKA	99	—	—	6
E 398-415	0.3	HKSGSSIGKAFTTTLKGA	97	—	1	5
E 426-443	0.4	WDFGSVGGVFTSVGKAVH	95	—	2	5
E 434-451	0.3	VFTSVGKAVHQVFGGAFR	96	—	1	5
E 442-459	0.2	VHQVFGGAFRSLFGGMSW	97	—	1	2
E 450-467	0.1	FRSLFGGMSWITQGLLGA	>99.3	—	—	3
E 458-474	0.1	SWITQGLLGALLLWMGI	>99	—	—	4
E 465-482	0.1	LGALLLWMGINARDRSIA	99	—	—	7
E 486-501	0.2	LAVGGVLLFLSVNVHA	98	—	—	5
NS1 1-19	0.7	DTGCAIDISRQELRCGSGV	90	—	1	5
NS1 10-27	0.2	RQELRCGSGVFIHNDVEA	98	—	—	3

TABLE 11-continued

WNV HLA-restricted T-cell epitope peptides incidence and their variant incidence distribution. Entropy describing the diversity at the epitope peptide sites is also indicated.

		ELISpot positive peptides		Variant peptides [c]		
Protein & position	Entropy [a]	Peptide sequence	Incidence (%) [b]	>10% (#)	1-10% (#)	<1% (#)
NS1 18-35	0.7	GVFIHNDVEAWMDRYKYY	89	—	2	5
NS1 161-178	0.9	GLTSTRMFLKVRESNTTE	87	—	3	6
NS1 205-221	0.8	RLNDTWKLERAVLGEVK	87	—	3	2
NS1 228-245	0.8	THTLWGDGILESDLIIPV	87	—	2	5
NS1 236-251	0.7	ILESDLIIPVTLAGPR	89	—	2	5
NS1 270-287	0.6	EGRVEIDFDYCPGTTVTL	91	—	2	2
NS1 305-322	0.1	GKLITDWCCRSCTLPPLR	99	—	—	2
NS1 327-343	0.8	SGCWYGMEIRPQRHDEK	87	—	3	4
NS2a 6-23	0.4	IDPFQLGLLVVFLATQEV	94	—	1	3
NS2a 45-61	0.4	VFGGITYTDVLRYVILV	96	—	—	6
NS2a 52-66	0.3	TDVLRYVILVGAAFA	97	—	—	5
NS2a 65-81	0.5	FAESNSGGDVVHLALMA	92	—	1	1
NS2a 80-97	0.7	MATFKIQPVFMVASFLKA	89	—	1	5
NS2a 128-145	1	EIPDVLNSLAVAWMILRA	85	—	4	5
NS2a 136-154	0.7	LAVAWMILRAITFTTTSNV	88	—	1	5
NS2a 160-177	0.8	ALLTPGLRCLNLDVYRIL	87	—	2	4
NS2a 168-183	1	CLNLDVYRILLLMVGI	78	1(18%)	1	4
NS2a 211-229	1.3	GLFNPMILAAGLIACDPNR	75	1(12%)	3	3
NS2b 1-18	0.1	GWPATEVMTAVGLMFAIV	>99	—	—	1
NS2b 39-56	0.3	MFAAFVISGKSTDMWIER	94	—	1	1
NS2b 59-75	0.9	DISWESDAEITGSSERV	85	—	3	3
NS2b 84-101	0.5	NFQLMNDPGAPWKIWMLR	94	—	2	4
NS2b 107-124	0.6	ISAYTPWAILPSVVGFWI	88	—	2	1
NS2b 115-131	0.6	ILPSVVGFWITLQYTKR	89	—	1	2
NS3 1-15	0.5	GGVLWDTPSPKEYKK	94	—	2	3
NS3 6-23	0.5	DTPSPKEYKKGDTTTGVY	94	—	2	3
NS3 22-38	0.2	VYRIMTRGLLGSYQAGA	98	—	—	3
NS3 37-54	0.2	GAGVMVEGVFHTLWHTTK	97	—	1	2
NS3 45-59	0.1	VFHTLWHTTKGAALM	99	—	—	2
NS3 50-65	0.1	WHTTKGAALMSGEGRL	99	—	—	2
NS3 70-87	0.3	GSVKEDRLCYGGPWKLQH	97	—	1	3
NS3 78-95	0.8	CYGGPWKLQHKWNGQDEV	87	—	2	4
NS3 85-100	0.7	LQHKWNGQDEVQMIVV	88	—	1	4
NS3 91-107	0.7	GQDEVQMIVVEPGKNVK	88	—	1	4

TABLE 11-continued

WNV HLA-restricted T-cell epitope peptides incidence and their variant incidence distribution. Entropy describing the diversity at the epitope peptide sites is also indicated.

		ELISpot positive peptides		Variant peptides [c]		
Protein & position	Entropy [a]	Peptide sequence	Incidence (%) [b]	>10% (#)	1-10% (#)	<1% (#)
NS3 98-115	0.3	IVVEPGKNVKNVQTKPGV	97	—	1	3
NS3 138-155	0.1	PIVDKNGDVIGLYGNGVI	99	—	—	2
NS3 146-162	0.2	VIGLYGNGVIMPNGSYI	97	—	—	2
NS3 161-177	0.8	YISAIVQGERMDEPIPA	86	—	2	3
NS3 176-192	0.4	PAGFEPEMLRKKQITVL	95	—	—	7
NS3 183-200	0.1	MLRKKQITVLDLHPGAGK	99	—	—	2
NS3 199-216	0.6	GKTRRILPQIIKEAINRR	90	—	2	2
NS3 206-223	0.6	PQIIKEAINRRLRTAVLA	91	—	1	4
NS3 214-230	0.6	NRRLRTAVLAPTRVVAA	90	—	1	5
NS3 221-237	0.7	VLAPTRVVAAEMAEALR	90	—	1	5
NS3 228-243	0.6	VAAEMAEALRGLPIRY	90	—	2	2
NS3 241-258	1.1	IRYCITSAVPREHNGNEIV	81	—	3	5
NS3 249-266	1.1	PREHNGNEIVDVMCHATL	81	—	3	4
NS3 265-282	0	TLTHRLMSPHRVPNYNLF	100	—	—	—
NS3 304-321	0.3	KVELGEAAAIFMTATPPG	95	—	1	1
NS3 317-333	0.7	ATPPGTSDPFPESNSPI	87	1(11%)	—	4
NS3 324-340	0.9	DPFPESNSPISDLQTEI	86	—	2	6
NS3 336-352	0.6	LQTEIPDRAWNSGYEWI	90	—	2	3
NS3 343-360	1.3	RAWNSGYEWITEYTGKTV	69	1(23%)	2	1
NS3 359-376	0.1	TVWFVPSVKMGNEIALCL	99	—	—	2
NS3 413-430	0.2	EMGANFKASRVIDSRKSV	97	—	—	2
NS3 455-469	0.4	AAQRRGRIGRNPSQV	95	—	2	2
NS3 460-475	0.4	GRIGRNPSQVGDEYCY	95	—	2	2
NS3 474-489	0.4	CYGGHTNEDDSNFAHW	95	—	2	1
NS3 480-496	0.4	NEDDSNFAHWTEARIML	94	—	2	2
NS3 487-501	0.1	AHWTEARIMLDNINM	>99	—	—	1
NS3 492-509	0.6	ARIMLDNINMPNGLIAQF	89	—	2	2
NS3 500-517	0.6	NMPNGLIAQFYQPEREKV	90	—	2	1
NS3 515-532	0.2	EKVYTMDGEYRLRGEERK	97	—	—	4
NS3 523-539	0.5	EYRLRGEERKNFLELLR	92	—	1	2
NS3 530-547	0.4	ERKNFLELLRTADLPVWL	92	—	1	1
NS3 545-562	0.9	VWLAYKVAAAGVSYHDRR	87	—	2	5
NS3 560-574	0.5	DRRWCFDGPRTNTIL	91	—	1	2
NS3 565-582	0.1	FDGPRTNTILEDNNEVEV	99	—	—	2

TABLE 11-continued

WNV HLA-restricted T-cell epitope peptides incidence and their variant incidence distribution. Entropy describing the diversity at the epitope peptide sites is also indicated.

Protein & position	Entropy [a]	ELISpot positive peptides: Peptide sequence	Incidence (%) [b]	Variant peptides [c]: >10% (#)	1-10% (#)	<1% (#)
NS3 581-597	0.6	EVITKLGERKILRPRWI	91	—	1	4
NS3 588-605	0.6	ERKILRPRWIDARVYSDH	90	—	1	3
NS4a 1-17	0.8	SQIGLIEVLGKMPEHFM	87	—	2	3
NS4a 15-31	0.2	HFMGKTWEALDTMYVVA	97	—	1	1
NS4a 54-71	1.1	IALIALLSVMTMGVFFLL	80	1(11%)	2	4
NS4a 62-79	0.5	VMTMGVFFLLMQRKGIGK	92	—	1	2
NS4a 86-103	1	VLGVATFFCWMAEVPGTK	84	—	4	4
NS4b 30-47	0.5	GEFLLDLRPATAWSLYAV	92	—	1	3
NS4b 38-55	0.2	PATAWSLYAVTTAVLTPL	97	—	—	4
NS4b 63-80	0.2	DYINTSLTSINVQASALF	98	—	1	1
NS4b 87-103	0.3	PFVDVGVSALLLAAGCW	96	—	1	2
NS4b 101-118	0.8	GCWGQVTLTVTVTAATLL	87	—	2	4
NS4b 187-204	0.6	VVNPSVKTVREAGILITA	90	—	1	5
NS4b 201-218	0.5	LITAAAVTLWENGASSVW	91	—	1	3
NS4b 233-250	1.5	GWLSCLSITWTLIKNMEK	76	—	5	5
NS4b 241-255	1.3	TWTLIKNMEKPGLKR	79	—	4	5
NS5 115-132	0.1	LVQSYGWNIVTMKSGVDV	>99	—	—	1
NS5 145-162	0.7	CDIGESSSSAEVEEHRTI	88	—	2	1
NS5 153-168	0.7	SAEVEEHRTIRVLEMV	87	—	2	1
NS5 181-198	0.6	VKVLCPYMPKVIEKMELL	91	—	1	4
NS5 212-229	0.5	SRNSTHEMYWVSRASGNV	91	—	1	2
NS5 448-463	0.5	ECHTCIYNMMGKREKK	92	—	2	1
NS5 470-486	0.1	AKGSRAIWFMWLGARFL	>99	—	—	1
NS5 477-494	0	WFMWLGARFLEFEALGFL	100	—	—	—
NS5 598-615	0	REDQRGSGQVVTYALNTF	100	—	—	—
NS5 605-622	0.1	GQVVTYALNTFTNLAVQL	99	—	1	—
NS5 613-631	0.1	NTFTNLAVQLVRMMEGEGV	99	—	1	—
NS5 704-721	0	GWYDWQQVPFCSNHFTEL	100	—	—	—
NS5 755-772	0.1	DTACLAKSYAQMWLLLYF	>99	—	—	1
NS5 763-780	0.1	YAQMWLLLYFHRRDLRLM	>99	—	—	1
NS5 771-788	0	YFHRRDLRLMANAICSAV	100	—	—	—
NS5 791-808	0.7	NWVPTGRTTWSIHAGGEW	88	—	2	4

TABLE 11-continued

WNV HLA-restricted T-cell epitope peptides incidence and their variant incidence distribution. Entropy describing the diversity at the epitope peptide sites is also indicated.

		ELISpot positive peptides		Variant peptides [c]		
Protein & position	Entropy [a]	Peptide sequence	Incidence (%) [b]	>10% (#)	1-10% (#)	<1% (#)
NS5 828-843	1	WMEDKTPVEKWSDVPY	85	—	2	9
NS5 842-859	0.1	PYSGKREDIWCGSLIGTR	98	—	—	3
NS5 863-879	1.6	TWAENIQVAINQVRAII	71	1(12%)	3	10

[a] Entropy value indicating the diversity of the region in the protein alignment that contained the epitope peptide sequence.
[b] The percentage of sequences (round off to the nearest whole number) analyzed that contained the exact sequence of the epitope peptide. Those with >99% do not include 100%
[c] The fraction of the variants of the epitope peptide sequence, greater than 10%, 1-10%, and less than 1%. The actual percentage representation of the major variant sequence is shown for the 7 epitope peptides with a variant that represents greater than 10% of the WNV sequences analyzed (see also Table 4).

TABLE 12

List of highly conserved and specific sequences of *Flavivirus* species West Nile virus (WNV), dengue virus (DENV), yellow fever virus (YFV) and Japanese encephalitis virus (JEV). (SEQ ID NOs: 1-206, in the order as shown)

Virus	Protein	Conserved Virus Specific Sequence
Dengue Virus (DENV)	E	VLGSQEGAMH
	NS1	VHTWTEQYKFQ
		AVHADMGYWIES
		HTLWSNGVLES
		GPWHLGKLE
	NS3	GLYGNGVVT
		LTIMDLHPG
		LMRRGDLPVWL
	NS4a	QRTPQDNQL
	NS4b	PASAWTLYAVATT
		HYAIIGPGLQAKATREAQKR
		AAGIMKNPTVDGI
		FWNTTIAVS
	NS5	SGVEGEGLH
		IFKLTYQNKVV
		DQRGSGQVGTYGLNTFTNME
		PTSRTTWSIHA
Japanese Encephalitis Virus (JEV)	C	SVAMKHLTSFK
	NS1	GGITYTDLARYVVL
	NS2a	LDTYRIILL
		MAKKKGAVL
		GLALTSTGWFSPTTI
	NS2b	AAITGSSRRLDVKLD
	NS3	GKLTPYWGSV
		YGGPWRFDRKWNGT
		DVQVIVVEPGK
		SYVSAIVQG
		LRGLPVRYQTSAVQREHQGN
		ASAAQRRGRV
		LDNIHMPNGLV
		QLYGPEREKA
	NS4a	LATFFLWAAEV
		GTKIAGTLL
		ALLLMVVLIPEP
		AANEYGMLE
		SQAGSLFVLPRGVP
		TDLDLTVGLV
	NS5	ERENHLRGECHT
		LARAIIELTY
		EIVMKDGRS
		RARISPGAG

TABLE 12-continued

List of highly conserved and specific sequences of *Flavivirus* species West Nile virus (WNV), dengue virus (DENV), yellow fever virus (YFV) and Japanese encephalitis virus (JEV). (SEQ ID NOs: 1-206, in the order as shown)

Virus	Protein	Conserved Virus Specific Sequence
Yellow Fever Virus (YFV)	C	MSGRKAQGKTLG
	prM	WCPDSMEYNCPNLS
		GRMGERQLQ
		ALLVLAVGPA
	E	KCVTVMAPDKPSLDISL
		DLTLPWQSGS
		HLVEFEPPH
		VLIEVNPPFGDS
		GDSRLTYQWHKEGSSI
		RNMTMSMSMI
	NS1	KRELKCGDG
		KYSYYPEDPVKLASI
		EEGKCGLNSVDSL
		HEMWRSRADEINAI
		YQRGTHPFSRIRDGLQYGWKTWGK
		FSPGRKNGSFIIDGKSRKECPFSNRVWNS
		ILGAAVNGKKSAHGSPTFWMGSHEVNGTWM
		LDYKECEWP
		THTIGTSVEE
		MFMPRSIGGPVSSHN
		IPGYKVQTNGPWMQVPLEV
		CTMPPVSFHG
		DGCWYPMEIRP
	NS2a	HAVPFGLVSMMIA
		LLGAMLVGQVT
		NNGGDAMYMALIA
		GFGLRTLWSPRERLV
		KDTSMQKTIP
		GLTQPFLGLCA
	NS2b	SIPVNEALAA
		GVLAGLAFQ
		MENFLGPIAVGG
		LMMLVSVAG
		WEEEAEISGSS
		EQGEFKLLSE
		VMTSLALVGAA
	NS3	SGDVLWDIPTPK
		GIFQSTFLGASQ
		TFLGASQRGVGVAQGGVFHTM
		PSWASVKEDLVAYGGSWKL
		WDGEEEVQLIAA
		VVNVQTKPS
		NGGEIGAVAL
		YGNGILVGDNSFVSAISQTE
		PGAGKTRRFLPQILAECARR
		AECARRRLRTL
		RRRLRTLVL
		GLDVKFHTQAFSAHGSG
		MCHATLTYRMLEPTR
		AHFLDPASIAAR
		ARGWAAHRARANESATILMTATPP
		ATPPGTSDEFPHSNGEIEDVQTDIPSEPW
		WILADKRPTAWFLP
		AWFLPSIRA
		LPSIRAANVMAASLRKAGK
		IAEMGANLCV
		KVAIKGPLRISA
		GRIGRNPNRDGDSYYYSEPTSE
		NAHHVCWLEASMLLDN
		MLLDNMEVRGGMVAPLYG
		KTPVSPGEMRLRDDQR
		GLKTNDRKWCF
		LRPRWCDERVSSDQSAL
	NS4a	RAYRNALSMMPEAMT
	NS4b	AAWTINVGI
		LSPMLHHWIK
		MLHHWIKVEYGNLSLSGI
		QSASVLSFMDKG
		PFMKMNISV
		LILPGIKAQQSKL

TABLE 12-continued

List of highly conserved and specific sequences of *Flavivirus* species West Nile virus (WNV), dengue virus (DENV), yellow fever virus (YFV) and Japanese encephalitis virus (JEV). (SEQ ID NOs: 1-206, in the order as shown)

Virus	Protein	Conserved Virus Specific Sequence
		DIEEAPEMP
		LYEKKLALYL
		KKLALYLLLALSL
		SVAMCRTPFSL
		PLIEGNTSLLWNGPMAVSMTGVMRGN
	NS5	GKTLGEVWKRELNLLD
		ARRHLAEGKV
		HLAEGKVDT
		AEGKVDTGVAVSRG
		VAVSRGTAK
		GTAKLRWFHERGYVKLEGRV
		WCYYAAAQKE
		YAAAQKEVSGVKG
		EKWLACGVDNFC
		EKLELLQRRFG
		LLQRRFGGT
		TFTVNQTSRLLMRRMRRPTGKVT
		LPIGTRSVETDKGPL
		DNDNPYRTWHYCGSY
		IEEVTRMAMTD
		RMAMTDTTP
		KEKVDTRAKDPPAGTRKIM
		VVNRWLFRHL
		QWKTANEAVQDPKFWE
		KLSEFGKAKG
		EDHWASRENSGGG
		GLQYLGYVI
		EADLDDEQEI
		MEMTYKNKVVK
		YKNKVVKVLR
		YALNTITNLKVQL
		ALSHLNAMSK
		MSKVRKDISEWQPSK
		GRGRVSPGNGW
		ACLSKAYANM
		SKAYANMWSLMYFHKRDMRLLS
		KRQDKLCGSL
West Nile Virus (WNV)	prM	LVAAVIGWMLGSNTMQRV
	E	LATVSDLSTKAACPTM
		FACSTKAIGRTILKENIK
		GIDTNAYYVMTVGTKTFL
		TFLVHREWFMDLNLPW
		VTVNPFVSVATANAKVLI
		IVVGRGEQQINHHWHK
		LAVGGVLLFLSVNVHA
	NS1	DTGCAIDISRQELRCGSGV
		RSVSRLEHQMW
		GLTSTRMFLKVRESNTTE
		THTLWGDGILESDLIIPV
	NS2a	VFGGITYTDVLRYVILV
		TDVLRYVILVGAAFA
		MATFKIQPVFMVASFLKA
		EIPDVLNSLAVAWMILRA
		LAVAWMILRAITFTTTSNV
		ALLTPGLRCLNLDVYRIL
		CLNLDVYRILLLMVGI
		GLFNPMILAAGLIACDPNR
	NS2b	GWPATEVMTA
		PMTIAGLMF
		DISWESDAEITGSSERV
		NFQLMNDPGAPWKIWMLR
		ISAYTPWAILPSVVGFWI
		ILPSVVGFWITLQYTKR
	NS3	VYRIMTRGLLGSYQAGA
		WHTTKGAALMSGEGRLDPYWGSV
		GSVKEDRLCYGGPWKLQH
		CYGGPWKLQHKWNGQDEV
		LQHKWNGQDEVQMIVV
		IVVEPGKNVKNVQTKPGV
		YISAIVQGERMDEPIPA

TABLE 12-continued

List of highly conserved and specific sequences of *Flavivirus* species West Nile virus (WNV), dengue virus (DENV), yellow fever virus (YFV) and Japanese encephalitis virus (JEV). (SEQ ID NOs: 1-206, in the order as shown)

Virus	Protein	Conserved Virus Specific Sequence
		PAGFEPEMLRKKQITVL
		IRYQTSAVPREHNGNEIV
		DPFPESNSPISDLQTEIPDRAWN
		RVIDSRKSVKP
		CYGGHTNEDDSNFAHW
		NEDDSNFAHWTEARIML
		AHWTEARIMLDNINM
		ARIMLDNINMPNGLIAQF
		NMPNGLIAQFYQPEREKV
		EVITKLGERKILRPRWI
		ERKILRPRWIDARVYSDH
	NS4a	SQIGLIEVLGKMPEHFM
		HFMGKTWEALDTMYVVA
		IALIALLSVMTMGVFFLL
		VMTMGVFFLLMQRKGIGK
		VLGVATFFCWMAEVPGTK
	NS4b	PATAWSLYAVTTAVLTPL
		DYINTSLTSINVQASALF
		PFVDVGVSALLLAAGCW
		LITAAAVTLWENGASSVW
		GWLSCLSITWTLIKNMEK
		TWTLIKNMEKPGLKR
	NS5	GGAKGRTLGE
		VEDWLHRGP
		EREAHLRGEC

Example 8

[0062] Large-scale analysis of the T-cell epitopes of WNV by immunization of HLA transgenic mice with 452 overlapping peptides spanning the entire WNV proteome has resulted in the identification of 137 peptides that elicited 200 HLA-restricted IFN-γ T-cell responses in 6 HLA transgenic mice strains: 74 for class I A2, A24, and B7, and 126 for class II DR2, DR3, and DR4. The multiple HLA responses to some of the peptides can be attributed to peptide promiscuity in T-cell activation, and to multiple T-cell epitope sequences in the same peptide. Many of these T-cell epitope peptides are likely dominant immunogens in nature, whether conveyed by natural pathogens or vaccines. Several mechanism(s) by which exogenous peptide immunogens are presented by antigen presenting cells in both HLA class I and II pathways have been described (Ackerman and Cresswell, 2004; Giodini and Cresswell, 2008; Lindner and Unanue, 1996; Nygard et al., 1994; Pathak and Blum, 2000; Pinet et al., 1995). Mechanistic studies have also shown that exogenous peptides compete for the presentation of endogenous antigens to MHC II-restricted T cells (Adorini et al., 1991) and that both endogenously processed peptide and the corresponding exogenous peptide act as ligands for a T-cell receptor (Gyotoku et al., 1998). There is also an abundance of evidence that support the HLA-transgenic mouse model for the efficient identification of peptides that contain sequences recognized both by the HLA molecules of the transgenic mice and by antigen receptors of the mouse T cells (Sonderstrup et al., 1999; Taneja and David, 1999). However, a limitation of this and most studies of T-cell epitopes is that because of the complexity of peptide HLA-processing and T-cell receptor recognition, the specific minimal epitope sequences are not known. As pointed out by Niels Jerne in 1960 (Jerne, 1960) processed peptides that would be recognized by T cells in association with MHC molecules are what he termed "cryptotopes," hidden epitopes which become immunologically available only after cellular processing. "T cell epitopes" as is now commonly used, describes the peptide sequence of the original protein, not the form that it is recognized by the T cell. For this reason, we herein use the terms "T cell epitope peptide" or "T cell epitope determinant" to describe the 15-18 amino acid peptides that contain T-cell epitopes of unknown specific sequence. Moreover, this mouse data is not used to elucidate the functional properties of human T-cells because the mouse T cells are educated to the HLA transgene, and little is known of the nature of this response as compared to the response of naive human T cells. Thus, our basic interpretation of these studies is that they reveal WNV protein sequences that contain T-cell epitopes specific for the selected HLA molecules and T cell class I or class II activation, but not a more detailed understanding of the functional role of these sequences in pathogen infection of humans.

[0063] A remarkable finding was the extensive identity of WNV epitope peptide sequences with other flaviviruses. WNV are among the more highly conserved RNA viruses with an average peptide sequence conservance of about 92% in all WNV in the public databases. In this study, there were only 7 epitope peptides that differed in more than 10% of the corresponding database sequences. However, and importantly, only 51 of the 137 epitope peptide sequences were specific for WNV and the remaining 86 contained sequences of 9 or more amino acids that collectively were identical to at least 67 other flaviviruses. Moreover, the entire sequences of 19 WNV epitope peptides, chiefly of the E, NS3 and NS5 proteins, were present in 26 viruses. Additionally, immune relevant homologous sequences of 9 or more amino acids of the 86 shared WNV epitope peptides were commonly found in other flaviviruses, with as many as 45 to 62 WNV sequences in Murray Valley encephalitis, Japanese encephalitis, and Usutu viruses. These mainly included E 99-113 and/or representatives of 8 NS5 sequences that each was found in 32 to 62 other flaviviruses. While the data presented are specific for WNV and other similar flaviviruses, we expect that the same would be true to some extent among other groups of phylogenetically related flaviviruses, certainly the 4 serotypes of dengue which contain many sequences with inter-DENV identity (Khan et al., 2008). There is high probability that many of the homologous sequences would act as epitopes or as altered peptide ligands in the event of multiple *Flavivirus* infections or immunization followed by infection with similar flaviviruses.

[0064] These findings have strong bearing on the possible pathological consequences of exposure to altered peptide ligands. Many studies have shown that peptide analogs recognized by T-cell receptors are participants in many T cell biological phenomena with possible pathogenic consequences (reviewed in Sloan-Lancaster and Allen, 1996; Mongkolsapaya, J. 2003). The selection of evolutionarily conserved protein sequences has widely been considered important to vaccine design in order to limit the selective loss of immunity resulting from mutation and protein modification. However, as shown herein, in the evolution of viruses, conserved sequences can be present in many different forms in viruses of related species and our conclusion is that the selection of virus specific sequences should have precedance to conserved, non-virus specific sequences. These observations also question the use of "ChimeriVax" vaccines such as the use of a yellow fever virus vaccine platform to deliver the premembrane and envelope genes of WNV (Monath et al., 2006), which clearly would have the potential of exposing the vaccine recipient to a large number of altered peptide ligands in the event of infection by WNV or any other *Flavivirus*.

[0065] The methodology applied to this study of WNV sequences provides an experimental basis for identification of HLA-restricted T cell epitope peptides of any pathogen. The analysis of pathogen antigens may conveniently use the same overlapping peptides required for ELISpot analysis of peptide specific T cell activation, but experiments comparing peptide and DNA-encoded antigen shown in this study and other unpublished experiments uniformly suggest the preferred use of genetic immunogens as vaccines. Selection of T-cell epitope peptides for vaccine design would omit sequences that are highly conserved in other related viruses, and focus on pathogen-specific sequences present in 80% or more of all recorded evolutionary variants of the pathogen and have clustered or closely contiguous localization. Clustered epitopes has distinct advantages in the design of an epitope-based vaccine, including the retention of native sequences for the function of transporters associated with antigen processing (TAPs) (Niedermann, 2002) and for the flanking sequences that are reported to modulate epitope processing and function in the selection of immunodominant epitopes (Le Gall et al., 2007). We elsewhere describe the use of sequences of the lysososome-associated membrane protein (LAMP) in the vaccine construct to elicit enhanced antigen delivery to the MHC II compartment of antigen presenting cells (de Arruda et al., 2004; Marques et al., 2003; Ruff et al., 1997).

REFERENCES

[0066] The disclosure of each reference cited is expressly incorporated herein.

[0067] Ackerman, A. L. and Cresswell, P. (2004). Cellular mechanisms governing cross-presentation of exogenous antigens, Nat Immunol, 5, 678-84.

[0068] Adorini, L., Moreno, J., Momburg, F., Hammerling, G. J., Guery, J. C., Valli, A. and Fuchs, S. (1991). Exogenous peptides compete for the presentation of endogenous antigens to major histocompatibility complex class II-restricted T cells, J Exp Med, 174, 945-8.

[0069] Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990). Basic local alignment search tool, J Mol Biol, 215, 403-10.

[0070] BenMohamed, L., Krishnan, R., Longmate, J., Auge, C., Low, L., Primus, J. and Diamond, D. J. (2000). Induction of CTL response by a minimal epitope vaccine in HLA A*0201/DR1 transgenic mice: dependence on HLA class II restricted T(H) response, Hum Immunol, 61, 764-79.

[0071] Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N. and Bourne, P. E. (2000). The Protein Data Bank, Nucleic Acids Res, 28, 235-42.

[0072] Blaney, J. E., Jr., Nobusawa, E., Brehm, M. A., Bonneau, R. H., Mylin, L. M., Fu, T. M., Kawaoka, Y. and Tevethia, S. S. (1998). Immunization with a single major histocompatibility complex class I-restricted cytotoxic T-lymphocyte recognition epitope of herpes simplex virus type 2 confers protective immunity, J Virol, 72, 9567-74.

[0073] Brien, J. D., Uhrlaub, J. L. and Nikolich-Zugich, J. (2007). Protective capacity and epitope specificity of CD8 (+) T cells responding to lethal West Nile virus infection, Eur J Immunol, 37, 1855-63.

[0074] Castrucci, M. R., Hou, S., Doherty, P. C. and Kawaoka, Y. (1994). Protection against lethal lymphocytic choriomeningitis virus (LCMV) infection by immunization of mice with an influenza virus containing an LCMV epitope recognized by cytotoxic T lymphocytes, J Virol, 68, 3486-90.

[0075] Cheuk, E., D'Souza, C., Hu, N., Liu, Y., Lang, H. and Chamberlain, J. W. (2002). Human MHC class I transgenic mice deficient for H2 class I expression facilitate identification and characterization of new HLA class I-restricted viral T cell epitopes, J Immunol, 169, 5571-80.

[0076] Cope, A. P., Patel, S. D., Hall, F., Congia, M., Hubers, H. A., Verheijden, G. F., Boots, A. M., Menon, R., Trucco M., Rijnders, A. W. and Sonderstrup. G. (1999). T cell responses to a human cartilage autoantigen in the context of rheumatoid arthritis-associated and nonassociated HLA-DR4 alleles, Arthritis Rheum, 42, 1497-507.

[0077] Davis, C. T., Galbraith, S. E., Zhang, S., Whiteman, M. C., Li, L., Kinney, R. M. and Barrett, A. D. (2007). A combination of naturally occurring mutations in North American West Nile virus nonstructural protein genes and in the 3' untranslated region alters virus phenotype, J Virol, 81, 6111-6.

[0078] de Arruda, L. B., Chikhlikar, P. R., August, J. T. and Marques, E. T. (2004). DNA vaccine encoding human immunodeficiency virus-1 Gag, targeted to the major histocompatibility complex II compartment by lysosomal-associated membrane protein, elicits enhanced long-term memory response, immunology, 112, 126-33.

[0079] de la Rosa, G., Yanez-Mo, M., Samaneigo, R., Serrano-Gomez, D., Martinez-Munoz, L., Fernandez-Ruiz, E., Longo, N., Sanchez-Madrid, F., Corbi, A. L. and Sanchez-Mateos, P. (2005). Regulated recruitment of DC-SIGN to cell-cell contact regions during zymosan-induced human dendritic cell aggregation, J Leukoc Biol, 77, 699-709.

[0080] Del Val, M., Schlicht, H. J., Volkmer, H., Messerle, M., Reddehase, M. J. and Koszinowski, U. H. (1991). Protection against lethal cytomegalovirus infection by a recombinant vaccine containing a single nonameric T-cell epitope, J Virol, 65, 3641-6.

[0081] Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput, Nucleic Acids Res, 32, 1792-7.

[0082] Ferrante, A. and Gorski, J. (2007). Cooperativity of hydrophobic anchor interactions: evidence for epitope selection by MHC class II as a folding process, J Immunol, 178, 7181-9.

[0083] Fugger, L., Michie, S. A., Rulifson, I., Lock, C. B. and McDevitt, G. S. (1994). Expression of HLA-DR4 and human CD4 transgenes in mice determines the variable region beta-chain T-cell repertoire and mediates an ULA-DR-restricted immune response, Proc Natl Acad Sci S A, 9/, 6151-5.

[0084] Giodini, A. and Cresswell, P. (2008). Hsp90-mediated cytosolic refolding of exogenous proteins internalized by dendritic cells, EMBO J, 27, 201-11.

[0085] Goodridge, H. S., Simmons, R. M. and Underhill, D. M. (2007). Dectin-1 stimulation by Candida albicans yeast or zymosan triggers NEAT activation in macrophages and dendritic cells, J Immunol, 178, 3107-15.

[0086] Gyotoku, T., Fukui, Y. and Sasazuki, T. (1998). An endogenously processed self peptide and the corresponding exogenous peptide bound to the same MHC class II molecule could be distinct ligands for TCR with different kinetic stability, Eur J Immunol, 28, 4050-61.

[0087] Hu, N., D'Souza, C., Cheung, H., Lang, H., Cheuk, E. and Chamberlain, J. W. (2005). Highly conserved pattern of recognition of influenza A wild-type and variant CD8+ CTL epitopes in HLA-A2+ humans and transgenic HLA-A2+/H2 class I-deficient mice. Vaccine, 23, 5231-44.

[0088] Jerre, N. K. (1960). Immunological speculations, Annu Rev Microbiol, 14, 341-58.

[0089] Kessler, P. D., Podsakoff, G. M., Chen, X., McQuiston, S. A., Colosi, P. C., Matelis, L. A., Kurtzman, G. J. and Byrne, B. J. (1996). Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein., Proc Natl Acad Sci USA, 93, 14082-7.

[0090] Khan, A. M., Miotto, O., Nascimento, E. J., Srinivasan, K. N., Heiny, A. T., Zhang, G. L., Marques, E. T., Tan, T. W., Brusic, V., Salmon, J. and August, J. T. (2008). Conservation and variability of dengue virus proteins: implications for vaccine design, PLoS Negl Trop Dis, 2, e272.

[0091] Kuno, G., Chang, G. J., Tsuchiya, K. R., Karabatsos, N. and Cropp, C. B. (1998). Phylogeny of the genus *Flavivirus*, J Virol, 72, 73-83.

[0092] La. Posta, V. J., Auperin, D. D., Kamin-Lewis, R. and Cole, G. A. (1993). Cross-protection against lymphocytic choriomeningitis virus mediated by a CD4+ T-cell clone specific for an envelope glycoprotein epitope of Lassa virus, J Virol, 67, 3497-506.

[0093] Le Gall, S., Stamegna, P. and Walker, B. D. (2007). Portable flanking sequences modulate CTL epitope processing, J Clin invest, 117, 3563-75.

[0094] Lieberman, M. M., Clements, D. E., Ogata, S., Wang, G., Corpuz, G., Wong, T., Martyak, T., Gilson, L., Coller, B. A., Leung, J., Watts, D. M., Tesh, R. B., Siirin, M., Travassos da Rosa, A., Humphreys, T. and Weeks-Levy, C. (2007). Preparation and immunogenic properties of a recombinant West Nile subunit vaccine, Vaccine, 25, 414-23.

[0095] Lindner, R. and Unanue, E. R. (1996). Distinct antigen MHC class II-complexes generated by separate processing pathways, EMBO J, 15, 6910-20.

[0096] Loirat, D., Lemonnier, F. A. and Michel, M. L. (2000). Multiepitopic HLA-A*0201-restricted immune response against hepatitis B surface antigen after DNA-based immunization, J Immunol, 165, 4748-55.

[0097] Madsen, L., Labrecque, N., Engberg, J., Dicrich, A., Svejgaard, A., Benoist, C., Mathis, D. and Fugger, L. (1999). Mice lacking all conventional MHC class II genes, Proc Natl Acad Sci USA, 96, 10338-43.

[0098] Marques, E. T., Jr., Chikhlikar, P., de Arruda, L. B., Leao, I. C., Lu, Y., Wong, J., Chen, J. S., Byrne, B. and August, J. T. (2003). HIV-1 p55Gag encoded in the lysosome-associated membrane protein-1 as a DNA plasmid vaccine chimera is highly expressed, traffics to the major histocompatibility class II compartment, and elicits enhanced immune responses, J Biol Chem, 278, 37926-36.

[0099] McMurtrey, C. P., Lelic, A., Piazza, P., Chakrabarti, A. K., Yablonsky, E. J., Wahl, A., Bardet, W., Eckerd, A., Cook, R. L., Hess, R., Buchli, R., Loeb, M., Rinaldo, C. R., Bramson, J. and Hildebrand, W. H. (2008). Epitope discovery in West Nile virus infection: Identification and immune recognition of viral epitopes, Proc Natl Acad Sci USA, 105, 2981-6.

[0100] Miotto, O., Tan, T. W. and Brusic, V. (2008). Rule-based knowledge aggregation for large-scale protein sequence analysis of influenza A viruses, BMC Bioinformatics, 9 *Suppl* 1, S7.

[0101] Monath, T. P., Liu, J., Kanesa-Thasan, N., Myers, G. A., Nichols, R., Deary, A., McCarthy, K., Johnson, C., Ermak, T., Shin, S., Arroyo, J., Guirakhoo, F., Kennedy, J. S., Ennis, F. A., Green, S. and Bedford, P. (2006). A live, attenuated recombinant West Nile virus vaccine, Proc Natl Acad Sci USA, 103, 6694-9.

[0102] Mongkolsapaya, J., Dejnirattisai, W., Xu, X. N., Vasanawathana, S., Tangthawornchaikui, N., Chairunsri, A., Sawasdivorn, S., Duangchinda, T., Dong, T., Rowland-Jones, S., Yenchitsomanus, P. T., McMichael, A., Malasit, P. and Screaton, G. (2003). Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever, Nat Med, 9, 921-7.

[0103] Mongkolsapaya, J., Duangchinda, T., Dejnirattisai, W., Vasanawathana, S., Avirutnan., P., Jairungsri, A., Khemnu, N., Tangthawornchaikul, N., Chotiyarnwong, P., Sae-Jang, K., Koch, M., Jones, Y., McMichael, A., Xu, X., Malasit, P. and Screaton, G. (2006). T cell responses in dengue hemorrhagic fever: are cross-reactive T cells suboptimal?, J Immunol, 176, 3821-9.

[0104] Moudy, R. M., Meola, M. A., Morin, L. L., Ebel, G. D. and Kramer, L. D. (2007). A newly emergent genotype of West Nile virus is transmitted earlier and more efficiently by Culex mosquitoes, Am J Trop Med Hyg, 77, 365-70.

[0105] Niedermann, C. (2002). Immunological functions of the proteasome, Curr Top Microbiol Immunol, 268, 91-136.

[0106] Nygard, N. R., Giacoletto, K. S., Bono, C., Gorka, J., Kompelli, S. and Schwartz, B. D. (1994). Peptide binding to surface class II molecules is the major pathway of formation of immunogenic class II-peptide complexes for viable antigen presenting cells, J Immunol, 152, 1082-93.

[0107] Oukka, M., Manuguerra, J. C., Livaditis, N., Tourdot, S., Riche, N., Vergnon, I., Cordopatis, P. and Kosmatopoulos, K. (1996). Protection against lethal viral infection by vaccination with nonimmunodominant peptides, J Immunol, 157, 3039-45.

[0108] Pajot, A., Michel, M. L., Fazillcau, N., Pancre, V., Auriault, C., Ojcius, D. M., Lemonnier, F. A. and Lone, Y. C. (2004). A mouse model of human adaptive immune functions: HLA-A2.1-/HLA-DR1-transgenic H-2 class I-/class II-knockout mice, Eur J Immunol, 34, 3060-9.

[0109] Pajot, A., Michel, M. L., Mancini-Bourgine, M., Ungeheuer, M. N., Ojcius, D. M., Deng, Q., Lemonnier, F. A. and Lone, Y. C. (2006). Identification of novel HLA-DR1-restricted epitopes from the hepatitis B virus envelope protein in mice expressing HLA-DR1 and vaccinated human subjects, Microbes infect, 8, 2783-90.

[0110] Parsons, R., Lelic, A., Hayes, L., Carter, A., Marshall, L., Evelegh, C., Drebot, M., Andonova, M., McMurtrey, C., Hildebrand, W., Loeb, M. B. and Bramson, J. L. (2008). The memory T cell response to West Nile virus in symptomatic humans following natural infection is not influenced by age and is dominated by a restricted set of CD8+ T cell epitopes, J Immunol, 181, 1563-72.

[0111] Pascolo, S. (2005). HLA class I transgenic mice: development, utilisation and improvement, Expert Opin Biol Ther, 5, 919-38.

[0112] Pascolo, S., Bervas, N., tire, J. M., Smith, A. G., Lemonnier, F. A. and Perarnau, B. (1997). HLA-A2.1-restricted education and cytolytic activity of CD8(+) T lymphocytes from betα2 microglobulin (beta2m) HLA-A2.1 monochain transgenic H-2 Db beta2m double knockout mice, J Exp Med, 185, 2043-51.

[0113] Pathak, S. S. and Blum, J. S. (2000). Endocytic recycling is required for the presentation of an exogenous peptide via MHC class II molecules, Traffic, 1, 561-9.

[0114] Pinet, V., Vergelli, M., Martin, R., Bakke, O. and Long, E. O. (1995). Antigen presentation mediated by recycling of surface HLA-DR molecules, Nature, 375, 603-6.

[0115] Purtha, W. E., Myers, N., Mitaksov, V., Sitati, E., Connolly, J., Fremont, D. H., Hansen, T. H. and Diamond, M. S. (2007). Antigen-specific cytotoxic T lymphocytes protect against lethal West Nile virus encephalitis, Eur J Immunol, 37, 1845-54.

[0116] Richards, K. A., Chaves, F. A., Krafcik, F. R., Topham, D. J., Lazarski, C. A. and Sant, A. J. (2007). Direct ex vivo analyses of HLA-DR1 transgenic mice reveal an exceptionally broad pattern of immunodominance in the primary HLA-DR1-restricted CD4 T-cell response to influenza virus hemagglutinin, J Virol, 81, 7608-19.

[0117] Robinson, J., Waller, M. J., Fail, S. C. and Marsh, S. G. (2006). The IMGT/HLA and IPD databases, Hum Mutat, 27, 1192-9.

[0118] Roederer, M. and Koup, R. A. (2003). Optimized determination of T cell epitope responses, J Immunol Methods, 274, 221-8.

[0119] Rohrlich, P. S., Cardinaud, S., Firat, Lamari, M., Briand, P., Escriou, N. and Lemonnier, F. A. (2003). HLA-B*0702 transgenic, H-2 KbDb double-knockout mice: phenotypical and functional characterization in response to influenza virus, Int Immunol, 15, 765-72.

[0120] Rothman, A. L. (2004). Dengue: defining protective versus pathologic immunity, J Clin Invest, 113, 946-51.

[0121] Ruff, A. L., Guarnieri, F. G., Staveley-O'Carroll, K., Siliciano, R. F. and August, J. T. (1997). The enhanced immune response to the HIV gp160/LAMP chimeric gene product targeted to the lysosome membrane protein trafficking pathway, J Biol Chem, 272, 8671-8.

[0122] Screaton, G. and Mongkolsapaya, J. (2006). T cell responses and dengue haemorrhagic fever, Novartis Found Symp, 277, 164-71; discussion 171-6, 251-3.

[0123] Shannon, C. E. (1948). A mathematical theory of communication, Bell System Technical Journal, 27, 379-423 and 623-656.

[0124] Sloan-Lancaster, J. and Allen, P. M. (1996). Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology, Annu Rev Immunol, 14, 1-27.

[0125] Sonderstrup, G., Cope, A. P., Patel, S., Congia, M., Hain, N., Hall, F. C., Parry, S. L., Fugger, L. H., Michie, S. and McDevitt, H. O. (1999). HLA class II transgenic mice: models of the human CD4+ T-cell immune response, Immunol Rev, 172, 335-43.

[0126] Stemmer, C., Quesnel, A., Prevost-Blondel, A., Zimmermann, C., Muller, S., Briand, J. P. and Pircher, H. (1999). Protection against lymphocytic choriomeningitis virus infection induced by a reduced peptide bond analogue of the H-2 Db-restricted CD8(+) T cell epitope GP33, J Biol Chem, 274, 5550-6.

[0127] Strauss, G., Vignali, D. A., Sehonrich, G. and Hammerling, G. J. (1994). Negative and positive selection by HLA-DR3(DRw17) molecules in transgenic mice, Immunogenetics, 40, 104-8.

[0128] Taneja, V. and David, C. S. (1999). HLA class II transgenic mice as models of human diseases, Immunol Rev, 169, 67-79.

[0129] Tsuji, M., Bergmann, C. C., Takita-Sonoda, Y., Murata, K., Rodrigues, E. G., Nussenzweig, R. S. and Zavala, F. (1998). Recombinant Sindbis viruses expressing a cytotoxic T-lymphocyte epitope of a malaria parasite or of influenza virus elicit protection against the corresponding pathogen in mice, J Virol, 72, 6907-10.

[0130] Vandenbark, A. A., Rich, C., Mooney, J., Zamora, A., Wang, C., Huan, J., Fugger, L., Offner, H., Jones, R. and Burrows, G. G. (2003). Recombinant TCR ligand induces tolerance to myelin oligodendrocyte glycoprotein 35-55 peptide and reverses clinical and histological signs of chronic experimental autoimmune encephalomyelitis in HLA-DR2 transgenic mice, J Immunol, 171, 127-33.

[0131] Wang, Y., Lobigs, M., Lee, E., Koskinen, A. and Mullbacher, A. (2006). CD8(+) T cell-mediated immune responses in West Nile virus (Sarafend strain) encephalitis are independent of gamma interferon, J Gen Virol, 87, 3599-609.

[0132] Wheeler, D. L., Barrett, T., Benson, D. A., Bryant, S. H., Canese, K., Church, D. M., DiCuccio, M., Edgar, R., Federhen, S., Helmberg, W., Kenton, D. L., Khovayko, O., Lipman, D. J., Madden, T. L., Maglott, D. R., Ostell, J., Pontius, J. U., Pruitt, K. D., Schuler, G. D., Schriml, L. M., Sequeira, E., Sherry, S. T., Sirotkin, K., Starchenko, G., Suzek, T. O., Tatusov, R., Tatusova, T. A., Wagner, L. and Yaschenko, E. (2005). Database resources of the National Center for Biotechnology Information, Nucleic Acids Res, 33, D39-45.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 347

<210> SEQ ID NO 1
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 1

Val Leu Gly Ser Gln Glu Gly Ala Met His
1 5 10

<210> SEQ ID NO 2
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 2

Val His Thr Trp Thr Glu Gln Tyr Lys Phe Gln
1 5 10

<210> SEQ ID NO 3
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 3

Ala Val His Ala Asp Met Gly Tyr Trp Ile Glu Ser
1 5 10

<210> SEQ ID NO 4
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 4

His Thr Leu Trp Ser Asn Gly Val Leu Glu Ser
1 5 10

<210> SEQ ID NO 5
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 5

Gly Pro Trp His Leu Gly Lys Leu Glu
1 5

<210> SEQ ID NO 6
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 6

Gly Leu Tyr Gly Asn Gly Val Val Thr
1 5

<210> SEQ ID NO 7
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 7

-continued

Leu Thr Ile Met Asp Leu His Pro Gly
1 5

<210> SEQ ID NO 8
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 8

Leu Met Arg Arg Gly Asp Leu Pro Val Trp Leu
1 5 10

<210> SEQ ID NO 9
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 9

Gln Arg Thr Pro Gln Asp Asn Gln Leu
1 5

<210> SEQ ID NO 10
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 10

Pro Ala Ser Ala Trp Thr Leu Tyr Ala Val Ala Thr Thr
1 5 10

<210> SEQ ID NO 11
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 11

His Tyr Ala Ile Ile Gly Pro Gly Leu Gln Ala Lys Ala Thr Arg Glu
1 5 10 15

Ala Gln Lys Arg
20

<210> SEQ ID NO 12
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 12

Ala Ala Gly Ile Met Lys Asn Pro Thr Val Asp Gly Ile
1 5 10

<210> SEQ ID NO 13
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 13

Phe Trp Asn Thr Thr Ile Ala Val Ser
1 5

<210> SEQ ID NO 14
<211> LENGTH: 9
<212> TYPE: PRT

-continued

<213> ORGANISM: Flavirus

<400> SEQUENCE: 14

Ser Gly Val Glu Gly Glu Gly Leu His
1   5

<210> SEQ ID NO 15
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 15

Ile Phe Lys Leu Thr Tyr Gln Asn Lys Val Val
1   5   10

<210> SEQ ID NO 16
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 16

Asp Gln Arg Gly Ser Gly Gln Val Gly Thr Tyr Gly Leu Asn Thr Phe
1   5   10   15

Thr Asn Met Glu
            20

<210> SEQ ID NO 17
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 17

Pro Thr Ser Arg Thr Thr Trp Ser Ile His Ala
1   5   10

<210> SEQ ID NO 18
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 18

Ser Val Ala Met Lys His Leu Thr Ser Phe Lys
1   5   10

<210> SEQ ID NO 19
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 19

Gly Gly Ile Thr Tyr Thr Asp Leu Ala Arg Tyr Val Val Leu
1   5   10

<210> SEQ ID NO 20
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 20

Leu Asp Thr Tyr Arg Ile Ile Leu Leu
1   5

-continued

<210> SEQ ID NO 21
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 21

```
Met Ala Lys Lys Lys Gly Ala Val Leu
1               5
```

<210> SEQ ID NO 22
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 22

```
Gly Leu Ala Leu Thr Ser Thr Gly Trp Phe Ser Pro Thr Thr Ile
1               5                   10                  15
```

<210> SEQ ID NO 23
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 23

```
Ala Ala Ile Thr Gly Ser Ser Arg Arg Leu Asp Val Lys Leu Asp
1               5                   10                  15
```

<210> SEQ ID NO 24
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 24

```
Gly Lys Leu Thr Pro Tyr Trp Gly Ser Val
1               5                   10
```

<210> SEQ ID NO 25
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 25

```
Tyr Gly Gly Pro Trp Arg Phe Asp Arg Lys Trp Asn Gly Thr
1               5                   10
```

<210> SEQ ID NO 26
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 26

```
Asp Val Gln Val Ile Val Val Glu Pro Gly Lys
1               5                   10
```

<210> SEQ ID NO 27
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 27

```
Ser Tyr Val Ser Ala Ile Val Gln Gly
1               5
```

-continued

<210> SEQ ID NO 28
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 28

Leu Arg Gly Leu Pro Val Arg Tyr Gln Thr Ser Ala Val Gln Arg Glu
1 5 10 15

His Gln Gly Asn
20

<210> SEQ ID NO 29
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 29

Ala Ser Ala Ala Gln Arg Arg Gly Arg Val
1 5 10

<210> SEQ ID NO 30
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 30

Leu Asp Asn Ile His Met Pro Asn Gly Leu Val
1 5 10

<210> SEQ ID NO 31
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 31

Gln Leu Tyr Gly Pro Glu Arg Glu Lys Ala
1 5 10

<210> SEQ ID NO 32
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 32

Leu Ala Thr Phe Phe Leu Trp Ala Ala Glu Val
1 5 10

<210> SEQ ID NO 33
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 33

Gly Thr Lys Ile Ala Gly Thr Leu Leu
1 5

<210> SEQ ID NO 34
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 34

Ala Leu Leu Leu Met Val Val Leu Ile Pro Glu Pro

-continued

1 5 10

<210> SEQ ID NO 35
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 35

Ala Ala Asn Glu Tyr Gly Met Leu Glu
1 5

<210> SEQ ID NO 36
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 36

Ser Gln Ala Gly Ser Leu Phe Val Leu Pro Arg Gly Val Pro
1 5 10

<210> SEQ ID NO 37
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 37

Thr Asp Leu Asp Leu Thr Val Gly Leu Val
1 5 10

<210> SEQ ID NO 38
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 38

Glu Arg Glu Asn His Leu Arg Gly Glu Cys His Thr
1 5 10

<210> SEQ ID NO 39
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 39

Leu Ala Arg Ala Ile Ile Glu Leu Thr Tyr
1 5 10

<210> SEQ ID NO 40
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 40

Glu Ile Val Met Lys Asp Gly Arg Ser
1 5

<210> SEQ ID NO 41
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 41

Arg Ala Arg Ile Ser Pro Gly Ala Gly

-continued

1 5

<210> SEQ ID NO 42
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 42

Met Ser Gly Arg Lys Ala Gln Gly Lys Thr Leu Gly
1 5 10

<210> SEQ ID NO 43
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 43

Trp Cys Pro Asp Ser Met Glu Tyr Asn Cys Pro Asn Leu Ser
1 5 10

<210> SEQ ID NO 44
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 44

Gly Arg Met Gly Glu Arg Gln Leu Gln
1 5

<210> SEQ ID NO 45
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 45

Ala Leu Leu Val Leu Ala Val Gly Pro Ala
1 5 10

<210> SEQ ID NO 46
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 46

Lys Cys Val Thr Val Met Ala Pro Asp Lys Pro Ser Leu Asp Ile Ser
1 5 10 15

Leu

<210> SEQ ID NO 47
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 47

Asp Leu Thr Leu Pro Trp Gln Ser Gly Ser
1 5 10

<210> SEQ ID NO 48
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 48

-continued

```
His Leu Val Glu Phe Glu Pro Pro His
1               5


<210> SEQ ID NO 49
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 49

Val Leu Ile Glu Val Asn Pro Pro Phe Gly Asp Ser
1               5                   10


<210> SEQ ID NO 50
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 50

Gly Asp Ser Arg Leu Thr Tyr Gln Trp His Lys Glu Gly Ser Ser Ile
1               5                   10                  15


<210> SEQ ID NO 51
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 51

Arg Asn Met Thr Met Ser Met Ser Met Ile
1               5                   10


<210> SEQ ID NO 52
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 52

Lys Arg Glu Leu Lys Cys Gly Asp Gly
1               5


<210> SEQ ID NO 53
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 53

Lys Tyr Ser Tyr Tyr Pro Glu Asp Pro Val Lys Leu Ala Ser Ile
1               5                   10                  15


<210> SEQ ID NO 54
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 54

Glu Glu Gly Lys Cys Gly Leu Asn Ser Val Asp Ser Leu
1               5                   10


<210> SEQ ID NO 55
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 55
```

-continued

His Glu Met Trp Arg Ser Arg Ala Asp Glu Ile Asn Ala Ile
1 5 10

<210> SEQ ID NO 56
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 56

Tyr Gln Arg Gly Thr His Pro Phe Ser Arg Ile Arg Asp Gly Leu Gln
1 5 10 15

Tyr Gly Trp Lys Thr Trp Gly Lys
20

<210> SEQ ID NO 57
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 57

Phe Ser Pro Gly Arg Lys Asn Gly Ser Phe Ile Ile Asp Gly Lys Ser
1 5 10 15

Arg Lys Glu Cys Pro Phe Ser Asn Arg Val Trp Asn Ser
20 25

<210> SEQ ID NO 58
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 58

Ile Leu Gly Ala Ala Val Asn Gly Lys Lys Ser Ala His Gly Ser Pro
1 5 10 15

Thr Phe Trp Met Gly Ser His Glu Val Asn Gly Thr Trp Met
20 25 30

<210> SEQ ID NO 59
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 59

Leu Asp Tyr Lys Glu Cys Glu Trp Pro
1 5

<210> SEQ ID NO 60
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 60

Thr His Thr Ile Gly Thr Ser Val Glu Glu
1 5 10

<210> SEQ ID NO 61
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 61

Met Phe Met Pro Arg Ser Ile Gly Gly Pro Val Ser Ser His Asn

-continued

```
1               5                   10                  15


<210> SEQ ID NO 62
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 62

Ile Pro Gly Tyr Lys Val Gln Thr Asn Gly Pro Trp Met Gln Val Pro
1               5                   10                  15

Leu Glu Val


<210> SEQ ID NO 63
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 63

Cys Thr Met Pro Pro Val Ser Phe His Gly
1               5                   10


<210> SEQ ID NO 64
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 64

Asp Gly Cys Trp Tyr Pro Met Glu Ile Arg Pro
1               5                   10


<210> SEQ ID NO 65
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 65

His Ala Val Pro Phe Gly Leu Val Ser Met Met Ile Ala
1               5                   10


<210> SEQ ID NO 66
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 66

Leu Leu Gly Ala Met Leu Val Gly Gln Val Thr
1               5                   10


<210> SEQ ID NO 67
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 67

Asn Asn Gly Gly Asp Ala Met Tyr Met Ala Leu Ile Ala
1               5                   10


<210> SEQ ID NO 68
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 68
```

-continued

Gly Phe Gly Leu Arg Thr Leu Trp Ser Pro Arg Glu Arg Leu Val
1   5   10   15

<210> SEQ ID NO 69
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 69

Lys Asp Thr Ser Met Gln Lys Thr Ile Pro
1   5   10

<210> SEQ ID NO 70
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 70

Gly Leu Thr Gln Pro Phe Leu Gly Leu Cys Ala
1   5   10

<210> SEQ ID NO 71
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 71

Ser Ile Pro Val Asn Glu Ala Leu Ala Ala
1   5   10

<210> SEQ ID NO 72
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 72

Gly Val Leu Ala Gly Leu Ala Phe Gln
1   5

<210> SEQ ID NO 73
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 73

Met Glu Asn Phe Leu Gly Pro Ile Ala Val Gly Gly
1   5   10

<210> SEQ ID NO 74
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 74

Leu Met Met Leu Val Ser Val Ala Gly
1   5

<210> SEQ ID NO 75
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 75

-continued

```
Trp Glu Glu Glu Ala Glu Ile Ser Gly Ser Ser
1               5                   10


<210> SEQ ID NO 76
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 76

Glu Gln Gly Glu Phe Lys Leu Leu Ser Glu
1               5                   10


<210> SEQ ID NO 77
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 77

Val Met Thr Ser Leu Ala Leu Val Gly Ala Ala
1               5                   10


<210> SEQ ID NO 78
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 78

Ser Gly Asp Val Leu Trp Asp Ile Pro Thr Pro Lys
1               5                   10


<210> SEQ ID NO 79
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 79

Gly Ile Phe Gln Ser Thr Phe Leu Gly Ala Ser Gln
1               5                   10


<210> SEQ ID NO 80
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 80

Thr Phe Leu Gly Ala Ser Gln Arg Gly Val Gly Val Ala Gln Gly Gly
1               5                   10                  15

Val Phe His Thr Met
            20


<210> SEQ ID NO 81
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 81

Pro Ser Trp Ala Ser Val Lys Glu Asp Leu Val Ala Tyr Gly Gly Ser
1               5                   10                  15

Trp Lys Leu


<210> SEQ ID NO 82
```

-continued

<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 82

Trp Asp Gly Glu Glu Glu Val Gln Leu Ile Ala Ala
1 5 10

<210> SEQ ID NO 83
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 83

Val Val Asn Val Gln Thr Lys Pro Ser
1 5

<210> SEQ ID NO 84
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 84

Asn Gly Gly Glu Ile Gly Ala Val Ala Leu
1 5 10

<210> SEQ ID NO 85
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 85

Tyr Gly Asn Gly Ile Leu Val Gly Asp Asn Ser Phe Val Ser Ala Ile
1 5 10 15

Ser Gln Thr Glu
20

<210> SEQ ID NO 86
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 86

Pro Gly Ala Gly Lys Thr Arg Arg Phe Leu Pro Gln Ile Leu Ala Glu
1 5 10 15

Cys Ala Arg Arg
20

<210> SEQ ID NO 87
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 87

Ala Glu Cys Ala Arg Arg Arg Leu Arg Thr Leu
1 5 10

<210> SEQ ID NO 88
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 88

-continued

```
Arg Arg Arg Leu Arg Thr Leu Val Leu
1               5


<210> SEQ ID NO 89
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 89

Gly Leu Asp Val Lys Phe His Thr Gln Ala Phe Ser Ala His Gly Ser
1               5                   10                  15

Gly


<210> SEQ ID NO 90
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 90

Met Cys His Ala Thr Leu Thr Tyr Arg Met Leu Glu Pro Thr Arg
1               5                   10                  15


<210> SEQ ID NO 91
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 91

Ala His Phe Leu Asp Pro Ala Ser Ile Ala Ala Arg
1               5                   10


<210> SEQ ID NO 92
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 92

Ala Arg Gly Trp Ala Ala His Arg Ala Arg Ala Asn Glu Ser Ala Thr
1               5                   10                  15

Ile Leu Met Thr Ala Thr Pro Pro
            20


<210> SEQ ID NO 93
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 93

Ala Thr Pro Pro Gly Thr Ser Asp Glu Phe Pro His Ser Asn Gly Glu
1               5                   10                  15

Ile Glu Asp Val Gln Thr Asp Ile Pro Ser Glu Pro Trp
            20                  25


<210> SEQ ID NO 94
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 94

Trp Ile Leu Ala Asp Lys Arg Pro Thr Ala Trp Phe Leu Pro
1               5                   10
```

-continued

<210> SEQ ID NO 95
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 95

Ala Trp Phe Leu Pro Ser Ile Arg Ala
1   5

<210> SEQ ID NO 96
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 96

Leu Pro Ser Ile Arg Ala Ala Asn Val Met Ala Ala Ser Leu Arg Lys
1   5   10   15

Ala Gly Lys

<210> SEQ ID NO 97
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 97

Ile Ala Glu Met Gly Ala Asn Leu Cys Val
1   5   10

<210> SEQ ID NO 98
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 98

Lys Val Ala Ile Lys Gly Pro Leu Arg Ile Ser Ala
1   5   10

<210> SEQ ID NO 99
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 99

Gly Arg Ile Gly Arg Asn Pro Asn Arg Asp Gly Asp Ser Tyr Tyr Tyr
1   5   10   15

Ser Glu Pro Thr Ser Glu
            20

<210> SEQ ID NO 100
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 100

Asn Ala His His Val Cys Trp Leu Glu Ala Ser Met Leu Leu Asp Asn
1   5   10   15

<210> SEQ ID NO 101
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

-continued

<400> SEQUENCE: 101

Met Leu Leu Asp Asn Met Glu Val Arg Gly Gly Met Val Ala Pro Leu
1 5 10 15

Tyr Gly

<210> SEQ ID NO 102
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 102

Lys Thr Pro Val Ser Pro Gly Glu Met Arg Leu Arg Asp Asp Gln Arg
1 5 10 15

<210> SEQ ID NO 103
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 103

Gly Leu Lys Thr Asn Asp Arg Lys Trp Cys Phe
1 5 10

<210> SEQ ID NO 104
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 104

Leu Arg Pro Arg Trp Cys Asp Glu Arg Val Ser Ser Asp Gln Ser Ala
1 5 10 15

Leu

<210> SEQ ID NO 105
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 105

Arg Ala Tyr Arg Asn Ala Leu Ser Met Met Pro Glu Ala Met Thr
1 5 10 15

<210> SEQ ID NO 106
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 106

Ala Ala Trp Thr Val Tyr Val Gly Ile
1 5

<210> SEQ ID NO 107
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 107

Leu Ser Pro Met Leu His His Trp Ile Lys
1 5 10

-continued

<210> SEQ ID NO 108
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 108

Met Leu His His Trp Ile Lys Val Glu Tyr Gly Asn Leu Ser Leu Ser
1   5   10   15

Gly Ile

<210> SEQ ID NO 109
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 109

Gln Ser Ala Ser Val Leu Ser Phe Met Asp Lys Gly
1   5   10

<210> SEQ ID NO 110
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 110

Pro Phe Met Lys Met Asn Ile Ser Val
1   5

<210> SEQ ID NO 111
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 111

Leu Ile Leu Pro Gly Ile Lys Ala Gln Gln Ser Lys Leu
1   5   10

<210> SEQ ID NO 112
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 112

Asp Ile Glu Glu Ala Pro Glu Met Pro
1   5

<210> SEQ ID NO 113
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 113

Leu Tyr Glu Lys Lys Leu Ala Leu Tyr Leu
1   5   10

<210> SEQ ID NO 114
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 114

Lys Lys Leu Ala Leu Tyr Leu Leu Leu Ala Leu Ser Leu
1   5   10

-continued

<210> SEQ ID NO 115
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 115

Ser Val Ala Met Cys Arg Thr Pro Phe Ser Leu
1   5   10

<210> SEQ ID NO 116
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 116

Pro Leu Ile Glu Gly Asn Thr Ser Leu Leu Trp Asn Gly Pro Met Ala
1   5   10   15

Val Ser Met Thr Gly Val Met Arg Gly Asn
            20   25

<210> SEQ ID NO 117
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 117

Gly Lys Thr Leu Gly Glu Val Trp Lys Arg Glu Leu Asn Leu Leu Asp
1   5   10   15

<210> SEQ ID NO 118
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 118

Ala Arg Arg His Leu Ala Glu Gly Lys Val
1   5   10

<210> SEQ ID NO 119
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 119

His Leu Ala Glu Gly Lys Val Asp Thr
1   5

<210> SEQ ID NO 120
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 120

Ala Glu Gly Lys Val Asp Thr Gly Val Ala Val Ser Arg Gly
1   5   10

<210> SEQ ID NO 121
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 121

-continued

Val Ala Val Ser Arg Gly Thr Ala Lys
1 5

<210> SEQ ID NO 122
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 122

Gly Thr Ala Lys Leu Arg Trp Phe His Glu Arg Gly Tyr Val Lys Leu
1 5 10 15

Glu Gly Arg Val
20

<210> SEQ ID NO 123
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 123

Trp Cys Tyr Tyr Ala Ala Ala Gln Lys Glu
1 5 10

<210> SEQ ID NO 124
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 124

Tyr Ala Ala Ala Gln Lys Glu Val Ser Gly Val Lys Gly
1 5 10

<210> SEQ ID NO 125
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 125

Glu Lys Trp Leu Ala Cys Gly Val Asp Asn Phe Cys
1 5 10

<210> SEQ ID NO 126
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 126

Glu Lys Leu Glu Leu Leu Gln Arg Arg Phe Gly
1 5 10

<210> SEQ ID NO 127
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 127

Leu Leu Gln Arg Arg Phe Gly Gly Thr
1 5

<210> SEQ ID NO 128
<211> LENGTH: 23
<212> TYPE: PRT

-continued

<213> ORGANISM: Flavirus

<400> SEQUENCE: 128

Thr Phe Thr Val Asn Gln Thr Ser Arg Leu Leu Met Arg Arg Met Arg
1 5 10 15

Arg Pro Thr Gly Lys Val Thr
20

<210> SEQ ID NO 129
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 129

Leu Pro Ile Gly Thr Arg Ser Val Glu Thr Asp Lys Gly Pro Leu
1 5 10 15

<210> SEQ ID NO 130
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 130

Asp Asn Asp Asn Pro Tyr Arg Thr Trp His Tyr Cys Gly Ser Tyr
1 5 10 15

<210> SEQ ID NO 131
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 131

Ile Glu Glu Val Thr Arg Met Ala Met Thr Asp
1 5 10

<210> SEQ ID NO 132
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 132

Arg Met Ala Met Thr Asp Thr Thr Pro
1 5

<210> SEQ ID NO 133
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 133

Lys Glu Lys Val Asp Thr Arg Ala Lys Asp Pro Pro Ala Gly Thr Arg
1 5 10 15

Lys Ile Met

<210> SEQ ID NO 134
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 134

Val Val Asn Arg Trp Leu Phe Arg His Leu
1 5 10

-continued

<210> SEQ ID NO 135
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 135

Gln Trp Lys Thr Ala Asn Glu Ala Val Gln Asp Pro Lys Phe Trp Glu
1 5 10 15

<210> SEQ ID NO 136
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 136

Lys Leu Ser Glu Phe Gly Lys Ala Lys Gly
1 5 10

<210> SEQ ID NO 137
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 137

Glu Asp His Trp Ala Ser Arg Glu Asn Ser Gly Gly Gly
1 5 10

<210> SEQ ID NO 138
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 138

Gly Leu Gln Tyr Leu Gly Tyr Val Ile
1 5

<210> SEQ ID NO 139
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 139

Glu Ala Asp Leu Asp Asp Glu Gln Glu Ile
1 5 10

<210> SEQ ID NO 140
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 140

Met Glu Met Thr Tyr Lys Asn Lys Val Val Lys
1 5 10

<210> SEQ ID NO 141
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 141

Tyr Lys Asn Lys Val Val Lys Val Leu Arg
1 5 10

-continued

<210> SEQ ID NO 142
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 142

Tyr Ala Leu Asn Thr Ile Thr Asn Leu Lys Val Gln Leu
1   5   10

<210> SEQ ID NO 143
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 143

Ala Leu Ser His Leu Asn Ala Met Ser Lys
1   5   10

<210> SEQ ID NO 144
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 144

Met Ser Lys Val Arg Lys Asp Ile Ser Glu Trp Gln Pro Ser Lys
1   5   10   15

<210> SEQ ID NO 145
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 145

Gly Arg Gly Arg Val Ser Pro Gly Asn Gly Trp
1   5   10

<210> SEQ ID NO 146
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 146

Ala Cys Leu Ser Lys Ala Tyr Ala Asn Met
1   5   10

<210> SEQ ID NO 147
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 147

Ser Lys Ala Tyr Ala Asn Met Trp Ser Leu Met Tyr Phe His Lys Arg
1   5   10   15

Asp Met Arg Leu Leu Ser
20

<210> SEQ ID NO 148
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 148

-continued

Lys Arg Gln Asp Lys Leu Cys Gly Ser Leu
1 5 10

<210> SEQ ID NO 149
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 149

Leu Val Ala Ala Val Ile Gly Trp Met Leu Gly Ser Asn Thr Met Gln
1 5 10 15

Arg Val

<210> SEQ ID NO 150
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 150

Leu Ala Thr Val Ser Asp Leu Ser Thr Lys Ala Ala Cys Pro Thr Met
1 5 10 15

<210> SEQ ID NO 151
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 151

Phe Ala Cys Ser Thr Lys Ala Ile Gly Arg Thr Ile Leu Lys Glu Asn
1 5 10 15

Ile Lys

<210> SEQ ID NO 152
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 152

Gly Ile Asp Thr Asn Ala Tyr Tyr Val Met Thr Val Gly Thr Lys Thr
1 5 10 15

Phe Leu

<210> SEQ ID NO 153
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 153

Thr Phe Leu Val His Arg Glu Trp Phe Met Asp Leu Asn Leu Pro Trp
1 5 10 15

<210> SEQ ID NO 154
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 154

Val Thr Val Asn Pro Phe Val Ser Val Ala Thr Ala Asn Ala Lys Val
1 5 10 15

Leu Ile

-continued

<210> SEQ ID NO 155
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 155

Ile Val Val Gly Arg Gly Glu Gln Gln Ile Asn His His Trp His Lys
1   5   10   15

<210> SEQ ID NO 156
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 156

Leu Ala Val Gly Gly Val Leu Leu Phe Leu Ser Val Asn Val His Ala
1   5   10   15

<210> SEQ ID NO 157
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 157

Asp Thr Gly Cys Ala Ile Asp Ile Ser Arg Gln Glu Leu Arg Cys Gly
1   5   10   15

Ser Gly Val

<210> SEQ ID NO 158
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 158

Arg Ser Val Ser Arg Leu Glu His Gln Met Trp
1   5   10

<210> SEQ ID NO 159
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 159

Gly Leu Thr Ser Thr Arg Met Phe Leu Lys Val Arg Glu Ser Asn Thr
1   5   10   15

Thr Glu

<210> SEQ ID NO 160
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 160

Thr His Thr Leu Trp Gly Asp Gly Ile Leu Glu Ser Asp Leu Ile Ile
1   5   10   15

Pro Val

<210> SEQ ID NO 161
<211> LENGTH: 17
<212> TYPE: PRT

-continued

<213> ORGANISM: Flavirus

<400> SEQUENCE: 161

Val Phe Gly Gly Ile Thr Tyr Thr Asp Val Leu Arg Tyr Val Ile Leu
1 5 10 15

Val

<210> SEQ ID NO 162
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 162

Thr Asp Val Leu Arg Tyr Val Ile Leu Val Gly Ala Ala Phe Ala
1 5 10 15

<210> SEQ ID NO 163
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 163

Met Ala Thr Phe Lys Ile Gln Pro Val Phe Met Val Ala Ser Phe Leu
1 5 10 15

Lys Ala

<210> SEQ ID NO 164
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 164

Glu Ile Pro Asp Val Leu Asn Ser Leu Ala Val Ala Trp Met Ile Leu
1 5 10 15

Arg Ala

<210> SEQ ID NO 165
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 165

Leu Ala Val Ala Trp Met Ile Leu Arg Ala Ile Thr Phe Thr Thr Thr
1 5 10 15

Ser Asn Val

<210> SEQ ID NO 166
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 166

Ala Leu Leu Thr Pro Gly Leu Arg Cys Leu Asn Leu Asp Val Tyr Arg
1 5 10 15

Ile Leu

<210> SEQ ID NO 167
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

-continued

<400> SEQUENCE: 167

Cys Leu Asn Leu Asp Val Tyr Arg Ile Leu Leu Leu Met Val Gly Ile
1 5 10 15

<210> SEQ ID NO 168
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 168

Gly Leu Phe Asn Pro Met Ile Leu Ala Ala Gly Leu Ile Ala Cys Asp
1 5 10 15

Pro Asn Arg

<210> SEQ ID NO 169
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 169

Gly Trp Pro Ala Thr Glu Val Met Thr Ala
1 5 10

<210> SEQ ID NO 170
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 170

Pro Met Thr Ile Ala Gly Leu Met Phe
1 5

<210> SEQ ID NO 171
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 171

Asp Ile Ser Trp Glu Ser Asp Ala Glu Ile Thr Gly Ser Ser Glu Arg
1 5 10 15

Val

<210> SEQ ID NO 172
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 172

Asn Phe Gln Leu Met Asn Asp Pro Gly Ala Pro Trp Lys Ile Trp Met
1 5 10 15

Leu Arg

<210> SEQ ID NO 173
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 173

Ile Ser Ala Tyr Thr Pro Trp Ala Ile Leu Pro Ser Val Val Gly Phe
1 5 10 15

-continued

Trp Ile

<210> SEQ ID NO 174
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 174

Ile Leu Pro Ser Val Val Gly Phe Trp Ile Thr Leu Gln Tyr Thr Lys
1 5 10 15

Arg

<210> SEQ ID NO 175
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 175

Val Tyr Arg Ile Met Thr Arg Gly Leu Leu Gly Ser Tyr Gln Ala Gly
1 5 10 15

Ala

<210> SEQ ID NO 176
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 176

Trp His Thr Thr Lys Gly Ala Ala Leu Met Ser Gly Glu Gly Arg Leu
1 5 10 15

Asp Pro Tyr Trp Gly Ser Val
20

<210> SEQ ID NO 177
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 177

Gly Ser Val Lys Glu Asp Arg Leu Cys Tyr Gly Gly Pro Trp Lys Leu
1 5 10 15

Gln His

<210> SEQ ID NO 178
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 178

Cys Tyr Gly Gly Pro Trp Lys Leu Gln His Lys Trp Asn Gly Gln Asp
1 5 10 15

Glu Val

<210> SEQ ID NO 179
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 179

-continued

Leu Gln His Lys Trp Asn Gly Gln Asp Glu Val Gln Met Ile Val Val
1 5 10 15

<210> SEQ ID NO 180
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 180

Ile Val Val Glu Pro Gly Lys Asn Val Lys Asn Val Gln Thr Lys Pro
1 5 10 15

Gly Val

<210> SEQ ID NO 181
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 181

Tyr Ile Ser Ala Ile Val Gln Gly Glu Arg Met Asp Glu Pro Ile Pro
1 5 10 15

Ala

<210> SEQ ID NO 182
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 182

Pro Ala Gly Phe Glu Pro Glu Met Leu Arg Lys Lys Gln Ile Thr Val
1 5 10 15

Leu

<210> SEQ ID NO 183
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 183

Ile Arg Tyr Gln Thr Ser Ala Val Pro Arg Glu His Asn Gly Asn Glu
1 5 10 15

Ile Val

<210> SEQ ID NO 184
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 184

Asp Pro Phe Pro Glu Ser Asn Ser Pro Ile Ser Asp Leu Gln Thr Glu
1 5 10 15

Ile Pro Asp Arg Ala Trp Asn
20

<210> SEQ ID NO 185
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 185

-continued

Arg Val Ile Asp Ser Arg Lys Ser Val Lys Pro
1 5 10

<210> SEQ ID NO 186
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 186

Cys Tyr Gly Gly His Thr Asn Glu Asp Asp Ser Asn Phe Ala His Trp
1 5 10 15

<210> SEQ ID NO 187
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 187

Asn Glu Asp Asp Ser Asn Phe Ala His Trp Thr Glu Ala Arg Ile Met
1 5 10 15

Leu

<210> SEQ ID NO 188
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 188

Ala His Trp Thr Glu Ala Arg Ile Met Leu Asp Asn Ile Asn Met
1 5 10 15

<210> SEQ ID NO 189
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 189

Ala Arg Ile Met Leu Asp Asn Ile Asn Met Pro Asn Gly Leu Ile Ala
1 5 10 15

Gln Phe

<210> SEQ ID NO 190
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 190

Asn Met Pro Asn Gly Leu Ile Ala Gln Phe Tyr Gln Pro Glu Arg Glu
1 5 10 15

Lys Val

<210> SEQ ID NO 191
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 191

Glu Val Ile Thr Lys Leu Gly Glu Arg Lys Ile Leu Arg Pro Arg Trp
1 5 10 15

Ile

-continued

<210> SEQ ID NO 192
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 192

Glu Arg Lys Ile Leu Arg Pro Arg Trp Ile Asp Ala Arg Val Tyr Ser
1   5   10   15

Asp His

<210> SEQ ID NO 193
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 193

Ser Gln Ile Gly Leu Ile Glu Val Leu Gly Lys Met Pro Glu His Phe
1   5   10   15

Met

<210> SEQ ID NO 194
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 194

His Phe Met Gly Lys Thr Trp Glu Ala Leu Asp Thr Met Tyr Val Val
1   5   10   15

Ala

<210> SEQ ID NO 195
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 195

Ile Ala Leu Ile Ala Leu Leu Ser Val Met Thr Met Gly Val Phe Phe
1   5   10   15

Leu Leu

<210> SEQ ID NO 196
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 196

Val Met Thr Met Gly Val Phe Phe Leu Leu Met Gln Arg Lys Gly Ile
1   5   10   15

Gly Lys

<210> SEQ ID NO 197
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 197

Val Leu Gly Val Ala Thr Phe Phe Cys Trp Met Ala Glu Val Pro Gly
1   5   10   15

Thr Lys

-continued

<210> SEQ ID NO 198
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 198

Pro Ala Thr Ala Trp Ser Leu Tyr Ala Val Thr Thr Ala Val Leu Thr
1 5 10 15

Pro Leu

<210> SEQ ID NO 199
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 199

Asp Tyr Ile Asn Thr Ser Leu Thr Ser Ile Asn Val Gln Ala Ser Ala
1 5 10 15

Leu Phe

<210> SEQ ID NO 200
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 200

Pro Phe Val Asp Val Gly Val Ser Ala Leu Leu Leu Ala Ala Gly Cys
1 5 10 15

Trp

<210> SEQ ID NO 201
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 201

Leu Ile Thr Ala Ala Ala Val Thr Leu Trp Glu Asn Gly Ala Ser Ser
1 5 10 15

Val Trp

<210> SEQ ID NO 202
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 202

Gly Trp Leu Ser Cys Leu Ser Ile Thr Trp Thr Leu Ile Lys Asn Met
1 5 10 15

Glu Lys

<210> SEQ ID NO 203
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 203

Thr Trp Thr Leu Ile Lys Asn Met Glu Lys Pro Gly Leu Lys Arg
1 5 10 15

-continued

<210> SEQ ID NO 204
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 204

```
Gly Gly Ala Lys Gly Arg Thr Leu Gly Glu
1               5                   10
```

<210> SEQ ID NO 205
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 205

```
Val Glu Asp Trp Leu His Arg Gly Pro
1               5
```

<210> SEQ ID NO 206
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Flavirus

<400> SEQUENCE: 206

```
Glu Arg Glu Ala His Leu Arg Gly Glu Cys
1               5                   10
```

<210> SEQ ID NO 207
<211> LENGTH: 380
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 207

```
Met Ala Pro Arg Ser Ala Arg Arg Pro Leu Leu Leu Leu Leu Leu Leu
1               5                   10                  15

Leu Leu Leu Gly Leu Met His Cys Ala Ser Ala Ala Met Phe Met Val
            20                  25                  30

Lys Asn Gly Asn Gly Thr Ala Cys Ile Met Ala Asn Phe Ser Ala Ala
        35                  40                  45

Phe Ser Val Asn Tyr Asp Thr Lys Ser Gly Pro Lys Asn Met Thr Leu
    50                  55                  60

Asp Leu Pro Ser Asp Ala Thr Val Val Leu Asn Arg Ser Ser Cys Gly
65                  70                  75                  80

Lys Glu Asn Thr Ser Asp Pro Ser Leu Val Ile Ala Phe Gly Arg Gly
                85                  90                  95

His Thr Leu Thr Leu Asn Phe Thr Arg Asn Ala Thr Arg Tyr Ser Val
            100                 105                 110

Gln Leu Met Ser Phe Val Tyr Asn Leu Ser Asp Thr His Leu Phe Pro
        115                 120                 125

Asn Ala Ser Ser Lys Glu Ile Lys Thr Val Glu Ser Ile Thr Asp Ile
    130                 135                 140

Arg Ala Asp Ile Asp Lys Lys Tyr Arg Cys Val Ser Gly Thr Gln Val
145                 150                 155                 160

His Met Asn Asn Val Thr Val Thr Leu His Asp Ala Thr Ile Gln Ala
                165                 170                 175

Tyr Leu Ser Asn Ser Ser Phe Ser Arg Gly Glu Thr Arg Cys Glu Gln
            180                 185                 190
```

-continued

Asp Arg Pro Ser Pro Thr Thr Ala Pro Pro Ala Pro Pro Ser Pro Ser
195 200 205

Pro Ser Pro Val Pro Lys Ser Pro Ser Val Asp Lys Tyr Asn Val Ser
210 215 220

Gly Thr Asn Gly Thr Cys Leu Leu Ala Ser Met Gly Leu Gln Leu Asn
225 230 235 240

Leu Thr Tyr Glu Arg Lys Asp Asn Thr Thr Val Thr Arg Leu Leu Asn
245 250 255

Ile Asn Pro Asn Lys Thr Ser Ala Ser Gly Ser Cys Gly Ala His Leu
260 265 270

Val Thr Leu Glu Leu His Ser Glu Gly Thr Thr Val Leu Leu Phe Gln
275 280 285

Phe Gly Met Asn Ala Ser Ser Ser Arg Phe Phe Leu Gln Gly Ile Gln
290 295 300

Leu Asn Thr Ile Leu Pro Asp Ala Arg Asp Pro Ala Phe Lys Ala Ala
305 310 315 320

Asn Gly Ser Leu Arg Ala Leu Gln Ala Thr Val Gly Asn Ser Tyr Lys
325 330 335

Cys Asn Ala Glu Glu His Val Arg Val Thr Lys Ala Phe Ser Val Asn
340 345 350

Ile Phe Lys Val Trp Val Gln Ala Phe Lys Val Glu Gly Gly Gln Phe
355 360 365

Gly Ser Val Glu Glu Cys Leu Leu Asp Glu Asn Ser
370 375 380

<210> SEQ ID NO 208
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 208

Thr Leu Ile Pro Ile Ala Val Gly Gly Ala Leu Ala Gly Leu Val Leu
1 5 10 15

Ile Val Leu Ile Ala Tyr Leu Val Gly Arg Lys Arg Ser His Ala Gly
20 25 30

Tyr Gln Thr Ile
35

<210> SEQ ID NO 209
<211> LENGTH: 1140
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 209

atggcgcccc gcagcgcccg gcgacccctg ctgctgctac tgctgttgct gctgctcggc 60

ctcatgcatt gtgcgtcagc agcaatgttt atggtgaaaa atggcaacgg gaccgcgtgc 120

ataatggcca acttctctgc tgccttctca gtgaactacg acaccaagag tggccctaag 180

aacatgaccc ttgacctgcc atcagatgcc acagtggtgc tcaaccgcag ctcctgtgga 240

aaagagaaca cttctgaccc cagtctcgtg attgcttttg gaagaggaca tacactcact 300

ctcaatttca cgagaaatgc aacacgttac agcgtccagc tcatgagttt tgtttataac 360

ttgtcagaca cacacctttt ccccaatgcg agctccaaag aaatcaagac tgtggaatct 420

ataactgaca tcagggcaga tatagataaa aaatacagat gtgttagtgg cacccaggtc 480

-continued

```
cacatgaaca acgtgaccgt aacgctccat gatgccacca tccaggcgta cctttccaac    540

agcagcttca gccggggaga gacacgctgt gaacaagaca ggccttcccc aaccacagcg    600

cccccctgcgc cacccagccc ctcgccctca cccgtgccca agagcccctc tgtggacaag    660

tacaacgtga gcggcaccaa cgggacctgc ctgctggcca gcatggggct gcagctgaac    720

ctcacctatg agaggaagga caacacgacg gtgacaaggc ttctcaacat caaccccaac    780

aagacctcgg ccagcgggag ctgcggcgcc cacctggtga ctctggagct gcacagcgag    840

ggcaccaccg tcctgctctt ccagttcggg atgaatgcaa gttctagccg gtttttccta    900

caaggaatcc agttgaatac aattcttcct gacgccagag accctgcctt taaagctgcc    960

aacggctccc tgcgagcgct gcaggccaca gtcggcaatt cctacaagtg caacgcggag   1020

gagcacgtcc gtgtcacgaa ggcgttttca gtcaatatat tcaaagtgtg ggtccaggct   1080

ttcaaggtgg aaggtggcca gtttggctct gtggaggagt gtctgctgga cgagaacagc   1140
```

<210> SEQ ID NO 210
<211> LENGTH: 117
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 210

```
acgctgatcc ccatcgctgt gggtggtgcc ctggcggggc tggtcctcat cgtcctcatc     60

gcctacctcg tcggcaggaa gaggagtcac gcaggctacc agactatcta gggtacc       117
```

<210> SEQ ID NO 211
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 211

```
Gly Lys Ser Arg Ala Val Asn Met Leu Lys Arg Gly Met Pro Arg Val
1               5                   10                  15

Leu
```

<210> SEQ ID NO 212
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 212

```
Ser Thr Lys Ala Thr Arg Tyr Leu Val Lys Thr Glu Ser Trp Ile Leu
1               5                   10                  15

Arg
```

<210> SEQ ID NO 213
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 213

```
Leu Val Lys Thr Glu Ser Trp Ile Leu Arg Asn Pro Gly Tyr Ala Leu
1               5                   10                  15

Val Ala
```

<210> SEQ ID NO 214
<211> LENGTH: 17
<212> TYPE: PRT

-continued

<213> ORGANISM: Flavivirus

<400> SEQUENCE: 214

Leu Arg Asn Pro Gly Tyr Ala Leu Val Ala Ala Val Ile Gly Trp Met
1 5 10 15

Leu

<210> SEQ ID NO 215
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 215

Leu Val Ala Ala Val Ile Gly Trp Met Leu Gly Ser Asn Thr Met Gln
1 5 10 15

Arg Val

<210> SEQ ID NO 216
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 216

Met Leu Gly Ser Asn Thr Met Gln Arg Val Val Phe Val Val Leu Leu
1 5 10 15

Leu Leu

<210> SEQ ID NO 217
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 217

Arg Val Val Phe Val Val Leu Leu Leu Leu Val Ala Pro Ala Tyr Ser
1 5 10 15

<210> SEQ ID NO 218
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 218

Pro Thr Ile Asp Val Lys Met Met Asn Met Glu Ala Ala Asn Leu Ala
1 5 10 15

Glu Val

<210> SEQ ID NO 219
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 219

Ala Glu Val Arg Ser Tyr Cys Tyr Leu Ala Thr Val Ser Asp Leu Ser
1 5 10 15

Thr Lys

<210> SEQ ID NO 220
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

-continued

<400> SEQUENCE: 220

Leu Ala Thr Val Ser Asp Leu Ser Thr Lys Ala Ala Cys Pro Thr Met
1 5 10 15

<210> SEQ ID NO 221
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 221

Leu Ser Thr Lys Ala Ala Cys Pro Thr Met Gly Glu Ala His Asn Asp
1 5 10 15

Lys Arg

<210> SEQ ID NO 222
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 222

Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser Ile
1 5 10 15

<210> SEQ ID NO 223
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 223

Phe Ala Cys Ser Thr Lys Ala Ile Gly Arg Thr Ile Leu Lys Glu Asn
1 5 10 15

Ile Lys

<210> SEQ ID NO 224
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 224

Gly Arg Thr Ile Leu Lys Glu Asn Ile Lys Tyr Glu Val Ala Ile Phe
1 5 10 15

Val His

<210> SEQ ID NO 225
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 225

Ile Lys Tyr Glu Val Ala Ile Phe Val His Gly Pro Thr Thr Val Glu
1 5 10 15

Ser His

<210> SEQ ID NO 226
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 226

-continued

```
Pro Ala Ala Pro Ser Tyr Thr Leu Lys Leu Gly Glu Tyr Gly Glu Val
1               5                   10                  15

Thr Val


<210> SEQ ID NO 227
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 227

Gly Ile Asp Thr Asn Ala Tyr Tyr Val Met Thr Val Gly Thr Lys Thr
1               5                   10                  15

Phe Leu


<210> SEQ ID NO 228
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 228

Thr Phe Leu Val His Arg Glu Trp Phe Met Asp Leu Asn Leu Pro Trp
1               5                   10                  15


<210> SEQ ID NO 229
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 229

Thr Leu Met Glu Phe Glu Glu Pro His Ala Thr Lys Gln Ser Val Ile
1               5                   10                  15

Ala Leu


<210> SEQ ID NO 230
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 230

Glu Lys Leu Gln Leu Lys Gly Thr Thr Tyr Gly Val Cys Ser Lys Ala
1               5                   10                  15

Phe Lys


<210> SEQ ID NO 231
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 231

Val Thr Val Asn Pro Phe Val Ser Val Ala Thr Ala Asn Ala Lys Val
1               5                   10                  15

Leu Ile


<210> SEQ ID NO 232
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 232

Lys Val Leu Ile Glu Leu Glu Pro Pro Phe Gly Asp Ser Tyr Ile Val
```

-continued

1 5 10 15

Val

<210> SEQ ID NO 233
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 233

Ile Val Val Gly Arg Gly Glu Gln Gln Ile Asn His His Trp His Lys
1 5 10 15

<210> SEQ ID NO 234
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 234

Glu Gln Gln Ile Asn His His Trp His Lys Ser Gly Ser Ser Ile Gly
1 5 10 15

Lys Ala

<210> SEQ ID NO 235
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 235

His Lys Ser Gly Ser Ser Ile Gly Lys Ala Phe Thr Thr Thr Leu Lys
1 5 10 15

Gly Ala

<210> SEQ ID NO 236
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 236

Trp Asp Phe Gly Ser Val Gly Gly Val Phe Thr Ser Val Gly Lys Ala
1 5 10 15

Val His

<210> SEQ ID NO 237
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 237

Val Phe Thr Ser Val Gly Lys Ala Val His Gln Val Phe Gly Gly Ala
1 5 10 15

Phe Arg

<210> SEQ ID NO 238
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 238

Val His Gln Val Phe Gly Gly Ala Phe Arg Ser Leu Phe Gly Gly Met
1 5 10 15

-continued

Ser Trp

<210> SEQ ID NO 239
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 239

Phe Arg Ser Leu Phe Gly Gly Met Ser Trp Ile Thr Gln Gly Leu Leu
1 5 10 15

Gly Ala

<210> SEQ ID NO 240
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 240

Ser Trp Ile Thr Gln Gly Leu Leu Gly Ala Leu Leu Leu Trp Met Gly
1 5 10 15

Ile

<210> SEQ ID NO 241
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 241

Leu Gly Ala Leu Leu Leu Trp Met Gly Ile Asn Ala Arg Asp Arg Ser
1 5 10 15

Ile Ala

<210> SEQ ID NO 242
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 242

Leu Ala Val Gly Gly Val Leu Leu Phe Leu Ser Val Asn Val His Ala
1 5 10 15

<210> SEQ ID NO 243
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 243

Asp Thr Gly Cys Ala Ile Asp Ile Ser Arg Gln Glu Leu Arg Cys Gly
1 5 10 15

Ser Gly Val

<210> SEQ ID NO 244
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 244

Arg Gln Glu Leu Arg Cys Gly Ser Gly Val Phe Ile His Asn Asp Val
1 5 10 15

-continued

Glu Ala

<210> SEQ ID NO 245
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 245

Gly Val Phe Ile His Asn Asp Val Glu Ala Trp Met Asp Arg Tyr Lys
1 5 10 15

Tyr Tyr

<210> SEQ ID NO 246
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 246

Gly Leu Thr Ser Thr Arg Met Phe Leu Lys Val Arg Glu Ser Asn Thr
1 5 10 15

Thr Glu

<210> SEQ ID NO 247
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 247

Arg Leu Asn Asp Thr Trp Lys Leu Glu Arg Ala Val Leu Gly Glu Val
1 5 10 15

Lys

<210> SEQ ID NO 248
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 248

Thr His Thr Leu Trp Gly Asp Gly Ile Leu Glu Ser Asp Leu Ile Ile
1 5 10 15

Pro Val

<210> SEQ ID NO 249
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 249

Ile Leu Glu Ser Asp Leu Ile Ile Pro Val Thr Leu Ala Gly Pro Arg
1 5 10 15

<210> SEQ ID NO 250
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 250

Glu Gly Arg Val Glu Ile Asp Phe Asp Tyr Cys Pro Gly Thr Thr Val
1 5 10 15

Thr Leu

-continued

<210> SEQ ID NO 251
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 251

Gly Lys Leu Ile Thr Asp Trp Cys Cys Arg Ser Cys Thr Leu Pro Pro
1 5 10 15

Leu Arg

<210> SEQ ID NO 252
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 252

Ser Gly Cys Trp Tyr Gly Met Glu Ile Arg Pro Gln Arg His Asp Glu
1 5 10 15

Lys

<210> SEQ ID NO 253
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 253

Ile Asp Pro Phe Gln Leu Gly Leu Leu Val Val Phe Leu Ala Thr Gln
1 5 10 15

Glu Val

<210> SEQ ID NO 254
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 254

Val Phe Gly Gly Ile Thr Tyr Thr Asp Val Leu Arg Tyr Val Ile Leu
1 5 10 15

Val

<210> SEQ ID NO 255
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 255

Thr Asp Val Leu Arg Tyr Val Ile Leu Val Gly Ala Ala Phe Ala
1 5 10 15

<210> SEQ ID NO 256
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 256

Phe Ala Glu Ser Asn Ser Gly Gly Asp Val Val His Leu Ala Leu Met
1 5 10 15

Ala

-continued

<210> SEQ ID NO 257
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 257

Met Ala Thr Phe Lys Ile Gln Pro Val Phe Met Val Ala Ser Phe Leu
1 5 10 15

Lys Ala

<210> SEQ ID NO 258
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 258

Glu Ile Pro Asp Val Leu Asn Ser Leu Ala Val Ala Trp Met Ile Leu
1 5 10 15

Arg Ala

<210> SEQ ID NO 259
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 259

Leu Ala Val Ala Trp Met Ile Leu Arg Ala Ile Thr Phe Thr Thr Thr
1 5 10 15

Ser Asn Val

<210> SEQ ID NO 260
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 260

Ala Leu Leu Thr Pro Gly Leu Arg Cys Leu Asn Leu Asp Val Tyr Arg
1 5 10 15

Ile Leu

<210> SEQ ID NO 261
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 261

Cys Leu Asn Leu Asp Val Tyr Arg Ile Leu Leu Leu Met Val Gly Ile
1 5 10 15

<210> SEQ ID NO 262
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 262

Gly Leu Phe Asn Pro Met Ile Leu Ala Ala Gly Leu Ile Ala Cys Asp
1 5 10 15

Pro Asn Arg

-continued

<210> SEQ ID NO 263
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 263

Gly Trp Pro Ala Thr Glu Val Met Thr Ala Val Gly Leu Met Phe Ala
1   5   10   15

Ile Val

<210> SEQ ID NO 264
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 264

Met Phe Ala Ala Phe Val Ile Ser Gly Lys Ser Thr Asp Met Trp Ile
1   5   10   15

Glu Arg

<210> SEQ ID NO 265
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 265

Asp Ile Ser Trp Glu Ser Asp Ala Glu Ile Thr Gly Ser Ser Glu Arg
1   5   10   15

Val

<210> SEQ ID NO 266
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 266

Asn Phe Gln Leu Met Asn Asp Pro Gly Ala Pro Trp Lys Ile Trp Met
1   5   10   15

Leu Arg

<210> SEQ ID NO 267
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 267

Ile Ser Ala Tyr Thr Pro Trp Ala Ile Leu Pro Ser Val Val Gly Phe
1   5   10   15

Trp Ile

<210> SEQ ID NO 268
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 268

Ile Leu Pro Ser Val Val Gly Phe Trp Ile Thr Leu Gln Tyr Thr Lys
1   5   10   15

Arg

-continued

<210> SEQ ID NO 269
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 269

Gly Gly Val Leu Trp Asp Thr Pro Ser Pro Lys Glu Tyr Lys Lys
1 5 10 15

<210> SEQ ID NO 270
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 270

Asp Thr Pro Ser Pro Lys Glu Tyr Lys Lys Gly Asp Thr Thr Thr Gly
1 5 10 15

Val Tyr

<210> SEQ ID NO 271
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 271

Val Tyr Arg Ile Met Thr Arg Gly Leu Leu Gly Ser Tyr Gln Ala Gly
1 5 10 15

Ala

<210> SEQ ID NO 272
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 272

Gly Ala Gly Val Met Val Glu Gly Val Phe His Thr Leu Trp His Thr
1 5 10 15

Thr Lys

<210> SEQ ID NO 273
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 273

Val Phe His Thr Leu Trp His Thr Thr Lys Gly Ala Ala Leu Met
1 5 10 15

<210> SEQ ID NO 274
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 274

Trp His Thr Thr Lys Gly Ala Ala Leu Met Ser Gly Glu Gly Arg Leu
1 5 10 15

<210> SEQ ID NO 275
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

-continued

<400> SEQUENCE: 275

Gly Ser Val Lys Glu Asp Arg Leu Cys Tyr Gly Gly Pro Trp Lys Leu
1   5   10   15

Gln His

<210> SEQ ID NO 276
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 276

Cys Tyr Gly Gly Pro Trp Lys Leu Gln His Lys Trp Asn Gly Gln Asp
1   5   10   15

Glu Val

<210> SEQ ID NO 277
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 277

Leu Gln His Lys Trp Asn Gly Gln Asp Glu Val Gln Met Ile Val Val
1   5   10   15

<210> SEQ ID NO 278
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 278

Gly Gln Asp Glu Val Gln Met Ile Val Val Glu Pro Gly Lys Asn Val
1   5   10   15

Lys

<210> SEQ ID NO 279
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 279

Ile Val Val Glu Pro Gly Lys Asn Val Lys Asn Val Gln Thr Lys Pro
1   5   10   15

Gly Val

<210> SEQ ID NO 280
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 280

Pro Ile Val Asp Lys Asn Gly Asp Val Ile Gly Leu Tyr Gly Asn Gly
1   5   10   15

Val Ile

<210> SEQ ID NO 281
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

-continued

<400> SEQUENCE: 281

Val Ile Gly Leu Tyr Gly Asn Gly Val Ile Met Pro Asn Gly Ser Tyr
1 5 10 15

Ile

<210> SEQ ID NO 282
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 282

Tyr Ile Ser Ala Ile Val Gln Gly Glu Arg Met Asp Glu Pro Ile Pro
1 5 10 15

Ala

<210> SEQ ID NO 283
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 283

Pro Ala Gly Phe Glu Pro Glu Met Leu Arg Lys Lys Gln Ile Thr Val
1 5 10 15

Leu

<210> SEQ ID NO 284
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 284

Met Leu Arg Lys Lys Gln Ile Thr Val Leu Asp Leu His Pro Gly Ala
1 5 10 15

Gly Lys

<210> SEQ ID NO 285
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 285

Gly Lys Thr Arg Arg Ile Leu Pro Gln Ile Ile Lys Glu Ala Ile Asn
1 5 10 15

Arg Arg

<210> SEQ ID NO 286
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 286

Pro Gln Ile Ile Lys Glu Ala Ile Asn Arg Arg Leu Arg Thr Ala Val
1 5 10 15

Leu Ala

<210> SEQ ID NO 287
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

-continued

<400> SEQUENCE: 287

Asn Arg Arg Leu Arg Thr Ala Val Leu Ala Pro Thr Arg Val Val Ala
1 5 10 15

Ala

<210> SEQ ID NO 288
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 288

Val Leu Ala Pro Thr Arg Val Val Ala Ala Glu Met Ala Glu Ala Leu
1 5 10 15

Arg

<210> SEQ ID NO 289
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 289

Val Ala Ala Glu Met Ala Glu Ala Leu Arg Gly Leu Pro Ile Arg Tyr
1 5 10 15

<210> SEQ ID NO 290
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 290

Ile Arg Tyr Gln Thr Ser Ala Val Pro Arg Glu His Asn Gly Asn Glu
1 5 10 15

Ile Val

<210> SEQ ID NO 291
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 291

Pro Arg Glu His Asn Gly Asn Glu Ile Val Asp Val Met Cys His Ala
1 5 10 15

Thr Leu

<210> SEQ ID NO 292
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 292

Thr Leu Thr His Arg Leu Met Ser Pro His Arg Val Pro Asn Tyr Asn
1 5 10 15

Leu Phe

<210> SEQ ID NO 293
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

-continued

<400> SEQUENCE: 293

Lys Val Glu Leu Gly Glu Ala Ala Ala Ile Phe Met Thr Ala Thr Pro
1 5 10 15

Pro Gly

<210> SEQ ID NO 294
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 294

Ala Thr Pro Pro Gly Thr Ser Asp Pro Phe Pro Glu Ser Asn Ser Pro
1 5 10 15

Ile

<210> SEQ ID NO 295
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 295

Asp Pro Phe Pro Glu Ser Asn Ser Pro Ile Ser Asp Leu Gln Thr Glu
1 5 10 15

Ile

<210> SEQ ID NO 296
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 296

Leu Gln Thr Glu Ile Pro Asp Arg Ala Trp Asn Ser Gly Tyr Glu Trp
1 5 10 15

Ile

<210> SEQ ID NO 297
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 297

Arg Ala Trp Asn Ser Gly Tyr Glu Trp Ile Thr Glu Tyr Thr Gly Lys
1 5 10 15

Thr Val

<210> SEQ ID NO 298
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 298

Thr Val Trp Phe Val Pro Ser Val Lys Met Gly Asn Glu Ile Ala Leu
1 5 10 15

Cys Leu

<210> SEQ ID NO 299
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

-continued

<400> SEQUENCE: 299

Glu Met Gly Ala Asn Phe Lys Ala Ser Arg Val Ile Asp Ser Arg Lys
1 5 10 15

Ser Val

<210> SEQ ID NO 300
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 300

Ala Ala Gln Arg Arg Gly Arg Ile Gly Arg Asn Pro Ser Gln Val
1 5 10 15

<210> SEQ ID NO 301
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 301

Gly Arg Ile Gly Arg Asn Pro Ser Gln Val Gly Asp Glu Tyr Cys Tyr
1 5 10 15

<210> SEQ ID NO 302
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 302

Cys Tyr Gly Gly His Thr Asn Glu Asp Asp Ser Asn Phe Ala His Trp
1 5 10 15

<210> SEQ ID NO 303
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 303

Asn Glu Asp Asp Ser Asn Phe Ala His Trp Thr Glu Ala Arg Ile Met
1 5 10 15

Leu

<210> SEQ ID NO 304
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 304

Ala His Trp Thr Glu Ala Arg Ile Met Leu Asp Asn Ile Asn Met
1 5 10 15

<210> SEQ ID NO 305
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 305

Ala Arg Ile Met Leu Asp Asn Ile Asn Met Pro Asn Gly Leu Ile Ala
1 5 10 15

Gln Phe

-continued

<210> SEQ ID NO 306
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 306

Asn Met Pro Asn Gly Leu Ile Ala Gln Phe Tyr Gln Pro Glu Arg Glu
1 5 10 15

Lys Val

<210> SEQ ID NO 307
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 307

Glu Lys Val Tyr Thr Met Asp Gly Glu Tyr Arg Leu Arg Gly Glu Glu
1 5 10 15

Arg Lys

<210> SEQ ID NO 308
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 308

Glu Tyr Arg Leu Arg Gly Glu Glu Arg Lys Asn Phe Leu Glu Leu Leu
1 5 10 15

Arg

<210> SEQ ID NO 309
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 309

Glu Arg Lys Asn Phe Leu Glu Leu Leu Arg Thr Ala Asp Leu Pro Val
1 5 10 15

Trp Leu

<210> SEQ ID NO 310
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 310

Val Trp Leu Ala Tyr Lys Val Ala Ala Ala Gly Val Ser Tyr His Asp
1 5 10 15

Arg Arg

<210> SEQ ID NO 311
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 311

Asp Arg Arg Trp Cys Phe Asp Gly Pro Arg Thr Asn Thr Ile Leu
1 5 10 15

-continued

<210> SEQ ID NO 312
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 312

Phe Asp Gly Pro Arg Thr Asn Thr Ile Leu Glu Asp Asn Asn Glu Val
1 5 10 15

Glu Val

<210> SEQ ID NO 313
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 313

Glu Val Ile Thr Lys Leu Gly Glu Arg Lys Ile Leu Arg Pro Arg Trp
1 5 10 15

Ile

<210> SEQ ID NO 314
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 314

Glu Arg Lys Ile Leu Arg Pro Arg Trp Ile Asp Ala Arg Val Tyr Ser
1 5 10 15

Asp His

<210> SEQ ID NO 315
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 315

Ser Gln Ile Gly Leu Ile Glu Val Leu Gly Lys Met Pro Glu His Phe
1 5 10 15

Met

<210> SEQ ID NO 316
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 316

His Phe Met Gly Lys Thr Trp Glu Ala Leu Asp Thr Met Tyr Val Val
1 5 10 15

Ala

<210> SEQ ID NO 317
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 317

Ile Ala Leu Ile Ala Leu Leu Ser Val Met Thr Met Gly Val Phe Phe
1 5 10 15

Leu Leu

-continued

<210> SEQ ID NO 318
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 318

Val Met Thr Met Gly Val Phe Phe Leu Leu Met Gln Arg Lys Gly Ile
1 5 10 15

Gly Lys

<210> SEQ ID NO 319
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 319

Val Leu Gly Val Ala Thr Phe Phe Cys Trp Met Ala Glu Val Pro Gly
1 5 10 15

Thr Lys

<210> SEQ ID NO 320
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 320

Gly Glu Phe Leu Leu Asp Leu Arg Pro Ala Thr Ala Trp Ser Leu Tyr
1 5 10 15

Ala Val

<210> SEQ ID NO 321
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 321

Pro Ala Thr Ala Trp Ser Leu Tyr Ala Val Thr Thr Ala Val Leu Thr
1 5 10 15

Pro Leu

<210> SEQ ID NO 322
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 322

Asp Tyr Ile Asn Thr Ser Leu Thr Ser Ile Asn Val Gln Ala Ser Ala
1 5 10 15

Leu Phe

<210> SEQ ID NO 323
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 323

Pro Phe Val Asp Val Gly Val Ser Ala Leu Leu Leu Ala Ala Gly Cys
1 5 10 15

-continued

Trp

<210> SEQ ID NO 324
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 324

Gly Cys Trp Gly Gln Val Thr Leu Thr Val Thr Val Thr Ala Ala Thr
1 5 10 15

Leu Leu

<210> SEQ ID NO 325
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 325

Val Val Asn Pro Ser Val Lys Thr Val Arg Glu Ala Gly Ile Leu Ile
1 5 10 15

Thr Ala

<210> SEQ ID NO 326
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 326

Leu Ile Thr Ala Ala Ala Val Thr Leu Trp Glu Asn Gly Ala Ser Ser
1 5 10 15

Val Trp

<210> SEQ ID NO 327
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 327

Gly Trp Leu Ser Cys Leu Ser Ile Thr Trp Thr Leu Ile Lys Asn Met
1 5 10 15

Glu Lys

<210> SEQ ID NO 328
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 328

Thr Trp Thr Leu Ile Lys Asn Met Glu Lys Pro Gly Leu Lys Arg
1 5 10 15

<210> SEQ ID NO 329
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 329

Leu Val Gln Ser Tyr Gly Trp Asn Ile Val Thr Met Lys Ser Gly Val
1 5 10 15

Asp Val

-continued

<210> SEQ ID NO 330
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 330

Cys Asp Ile Gly Glu Ser Ser Ser Ser Ala Glu Val Glu Glu His Arg
1 5 10 15

Thr Ile

<210> SEQ ID NO 331
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 331

Ser Ala Glu Val Glu Glu His Arg Thr Ile Arg Val Leu Glu Met Val
1 5 10 15

<210> SEQ ID NO 332
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 332

Val Lys Val Leu Cys Pro Tyr Met Pro Lys Val Ile Glu Lys Met Glu
1 5 10 15

Leu Leu

<210> SEQ ID NO 333
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 333

Ser Arg Asn Ser Thr His Glu Met Tyr Trp Val Ser Arg Ala Ser Gly
1 5 10 15

Asn Val

<210> SEQ ID NO 334
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 334

Glu Cys His Thr Cys Ile Tyr Asn Met Met Gly Lys Arg Glu Lys Lys
1 5 10 15

<210> SEQ ID NO 335
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 335

Ala Lys Gly Ser Arg Ala Ile Trp Phe Met Trp Leu Gly Ala Arg Phe
1 5 10 15

Leu

<210> SEQ ID NO 336

-continued

<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 336

Trp Phe Met Trp Leu Gly Ala Arg Phe Leu Glu Phe Glu Ala Leu Gly
1 5 10 15

Phe Leu

<210> SEQ ID NO 337
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 337

Arg Glu Asp Gln Arg Gly Ser Gly Gln Val Val Thr Tyr Ala Leu Asn
1 5 10 15

Thr Phe

<210> SEQ ID NO 338
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 338

Gly Gln Val Val Thr Tyr Ala Leu Asn Thr Phe Thr Asn Leu Ala Val
1 5 10 15

Gln Leu

<210> SEQ ID NO 339
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 339

Asn Thr Phe Thr Asn Leu Ala Val Gln Leu Val Arg Met Met Glu Gly
1 5 10 15

Glu Gly Val

<210> SEQ ID NO 340
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 340

Gly Trp Tyr Asp Trp Gln Gln Val Pro Phe Cys Ser Asn His Phe Thr
1 5 10 15

Glu Leu

<210> SEQ ID NO 341
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 341

Asp Thr Ala Cys Leu Ala Lys Ser Tyr Ala Gln Met Trp Leu Leu Leu
1 5 10 15

Tyr Phe

-continued

<210> SEQ ID NO 342
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 342

Tyr Ala Gln Met Trp Leu Leu Leu Tyr Phe His Arg Arg Asp Leu Arg
1 5 10 15

Leu Met

<210> SEQ ID NO 343
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 343

Tyr Phe His Arg Arg Asp Leu Arg Leu Met Ala Asn Ala Ile Cys Ser
1 5 10 15

Ala Val

<210> SEQ ID NO 344
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 344

Asn Trp Val Pro Thr Gly Arg Thr Thr Trp Ser Ile His Ala Gly Gly
1 5 10 15

Glu Trp

<210> SEQ ID NO 345
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 345

Trp Met Glu Asp Lys Thr Pro Val Glu Lys Trp Ser Asp Val Pro Tyr
1 5 10 15

<210> SEQ ID NO 346
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 346

Pro Tyr Ser Gly Lys Arg Glu Asp Ile Trp Cys Gly Ser Leu Ile Gly
1 5 10 15

Thr Arg

<210> SEQ ID NO 347
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Flavivirus

<400> SEQUENCE: 347

Thr Trp Ala Glu Asn Ile Gln Val Ala Ile Asn Gln Val Arg Ala Ile
1 5 10 15

Ile

We claim:

1. A polypeptide comprising: one or more discontinuous segments of one or more proteins of a *Flavivirus*, said segments comprising at least 9 contiguous amino acid residues selected from SEQ ID NO: 1-206.

2. The polypeptide of claim **1** which further comprises: (a) a LAMP-1 lumenal sequence comprising SEQ ID NO: 207, and (b) a LAMP transmembrane and cytoplasmic tail comprising SEQ ID NO: 208, wherein the lumenal sequence is amino-terminal to the one or more discontinuous segments of the proteins of *Flavivirus* which are amino-terminal to the LAMP transmembrane and cytoplasmic tail.

3. The polypeptide of claim **1** wherein the segments are from a single *Flavivirus*.

4. The polypeptide of claim **1** wherein the segments are from a plurality of flaviviruses.

5. The polypeptide of claim **3** wherein the segments are from Yellow Fever Virus.

6. The polypeptide of claim **3** wherein the segments are from Dengue Virus.

7. The polypeptide of claim **3** wherein the segments are from West Nile Virus.

8. The polypeptide of claim **3** wherein the segments are from Japanese encephalitis virus.

9. A polynucleotide encoding the polypeptide of claim **1** or **2**.

10. The polynucleotide of claim **9** wherein codons encoding the polypeptide are optimized according to most frequent human codon usage.

11. The polynucleotide of claim **9** comprising SEQ ID NO: 209 encoding the LAMP-1 lumenal sequence and SEQ ID NO: 210 encoding the transmembrane and cytoplasmic tail of LAMP-1.

12. A nucleic acid vector which comprises the polynucleotide of claim **9**.

13. The nucleic acid vector of claim **12** which is a DNA virus.

14. The nucleic acid vector of claim **12** which is a RNA virus.

15. The nucleic acid vector of claim **12** which is a plasmid.

16. A host cell which comprises a nucleic acid vector of claim **12**.

17. A method of producing a polypeptide comprising, culturing a host cell according to claim **16** under conditions in which the host cell expresses the polypeptide.

18. The method of claim **17** further comprising, harvesting the peptide from the culture medium or host cells.

19. A method of producing a cellular vaccine comprising:
transfecting antigen presenting cells with a nucleic acid vector according to claim **11**, whereby the antigen presenting cells express the polypeptide.

20. The method of claim **19** wherein the antigen presenting cells are dendritic cells.

21. A method of making a vaccine, comprising: mixing together the polypeptide of claim **1** and an immune adjuvant.

22. The method of claim **21** wherein the adjuvant is selected from the group consisting of alum, lecithin, squalene, and a Toll-like receptors (TLRs) adaptor molecule.

23. A vaccine composition comprising the polypeptide of claim **1** or **2**.

24. A method of immunizing a human or other animal subject, comprising:
administering to the human or other animal subject a polypeptide of claim **1** or a nucleic acid vector according to claim **12** or a host cell according to claim **16**, in an amount effective to elicit *Flavivirus*-specific T cell activation.

25. The method of claim **24** further comprising administering to the subject a live or attenuated *Flavivirus* vaccine.

26. The method of claim **24** further comprising administering an immune adjuvant to the subject.

27. The method of claim **24** wherein the administration is oral, mucosal, nasal, intramuscular, intravenous, intradermal, intranasal, subcutaneous, or via electroporation.

28. A method of identifying a *Flavivirus*, comprising:
hybridizing a polynucleotide according to claim **9** or its complement to a *Flavivirus* genome, wherein hybridization of the genome to the polynucleotide indicates a species of the *Flavivirus*.

29. The method of claim **28** wherein the polynucleotide is from 15-90 nucleotides in length.

30. A method of identifying a *Flavivirus*, comprising:
contacting proteins from a virus-infected cell with an antibody which specifically binds to a polypeptide of claim **1**, wherein specific binding to the proteins indicates a species of *Flavivirus*.

31. A method of identifying a *Flavivirus*, comprising:
contacting a polypeptide of claim **1** with a blood sample from a patient, wherein binding of the polypeptide to an antibody in the blood sample or T cells in the blood sample indicates a species of *Flavivirus*.

* * * * *

专利名称(译)	用于疫苗和诊断用途的黄病毒种特异性肽标签		
公开(公告)号	US20130011427A1	公开(公告)日	2013-01-10
申请号	US13/516501	申请日	2010-12-16
[标]申请(专利权)人(译)	约翰霍普金斯大学		
申请(专利权)人(译)	约翰·霍普金斯大学		
当前申请(专利权)人(译)	八月，J·托马斯		
[标]发明人	AUGUST J THOMAS TAN TIN WEE KHAN ASIF MOHAMMAD		
发明人	AUGUST, J. THOMAS TAN, TIN WEE KHAN, ASIF MOHAMMAD		
IPC分类号	A61K39/12 C12N15/40 A61P37/04 C12N5/10 G01N33/53 C07K7/06 C12N15/63		
CPC分类号	A61K39/00 G01N2333/18 G01N33/56983 C07K14/005 C12N2770/24122 Y02A50/386 Y02A50/388 Y02A50/39 Y02A50/394 Y02A50/53 Y02A50/60		
优先权	61/287055 2009-12-16 US		
外部链接	Espacenet USPTO		

摘要(译)

黄病毒代表了日益严重的全球公共卫生问题，目前许多人没有预防和治疗配方。进化变化，全球变暖和广泛的动物宿主等因素的组合表明黄病毒株可能具有更大的分布和人类致病性。因此，需要更好地理解在人免疫应答中起作用的病毒蛋白质序列。报告的主要黄病毒序列的进化多样性，例如登革热病毒，黄热病病毒，日本脑炎病毒和西尼罗河病毒，用实验和生物信息学方法的组合进行分析。对所有报道的序列的分析揭示，由于与已知或预测的表位相对应，这些物种特异性肽标签是高度保守的并且是潜在的T细胞表位。这些肽标签与新一代疫苗和诊断应用的开发直接相关。