Update on flavivirus virulence studies

Alan D.T. Barrett

Department of Pathology,
Center for Tropical Diseases,
Sealy Center for Vaccine Development,
University of Texas Medical Branch

Important publications on West Nile

- *Viral Immunology*, Volume 13, 2000.
- *Emerging Infectious Diseases*, Volume 7, July-August, 2001.
- *Annals of the New York Academy of Sciences*, Volume 951, December 2001.
- *Current Topics in Microbiology and Immunology*, Volume 267, March 2002.

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Major Flavivirus Diseases

- Dengue
- Japanese encephalitis
- Tick-borne encephalitis
- West Nile
- Yellow fever

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West Nile virus

- Family: *Flaviviridae*
- Genus: *Flavivirus*
- Japanese encephalitis virus group

Cacipacore virus

Koutango virus

Japanese encephalitis virus

Murray Valley encephalitis virus
(Alfuy virus)

St. Louis encephalitis virus

Usutu virus

West Nile virus
(Kunjin virus)

Yaounde virus

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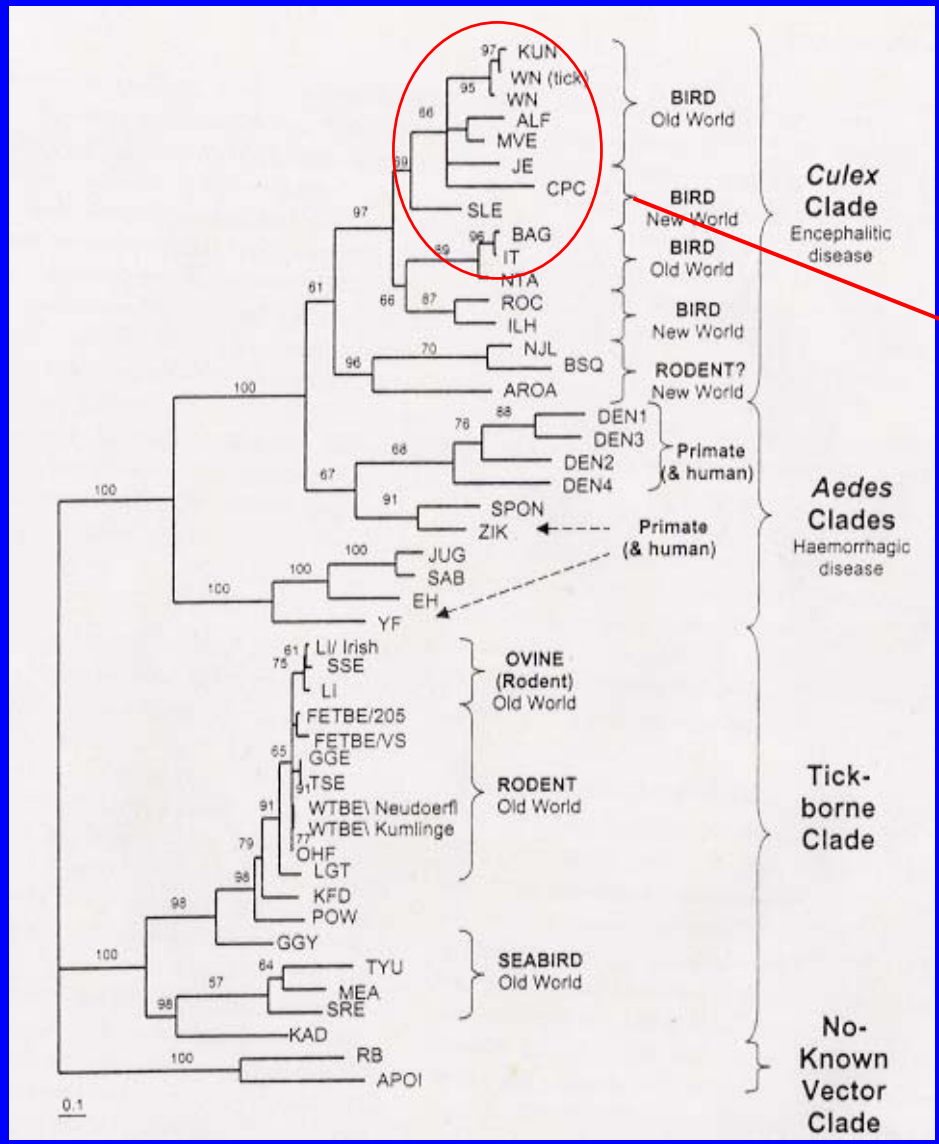
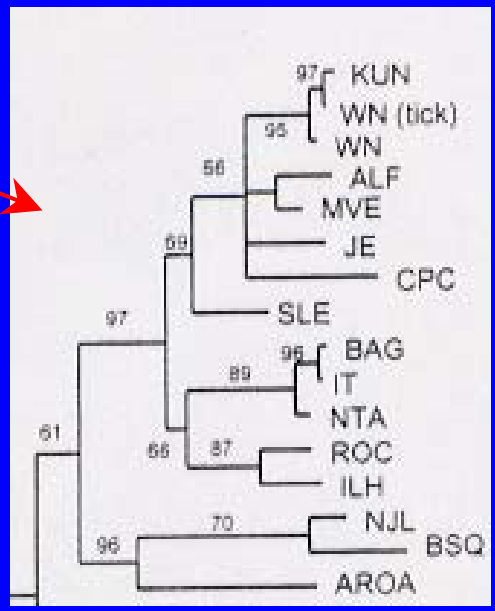
Phylogeny of the Flavivirus genus (Gaunt et al., 2001)





West Nile Virus Transmission Cycle

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Pathogenesis

- Virus infects host via mosquito bite.
- Multiplication in tissues and lymph nodes near site of entry.
- Virus moves to blood via lymphatics; viremia detected early in infection.
- Infection of central nervous system takes place.

How does West Nile virus invade the CNS?

Four mechanisms to explain entry into brain

→Neuronal route after infection of peripheral nerves.

→Virus enters brain via axonal transport through olfactory neurons.

→Virus crosses blood-brain barrier via replication in vascular endothelial cells in brain capillaries, transcytosis and release of virus into brain parenchyma.

→Diffusion of virus from vascular endothelial cells in situations where blood-brain barrier is leaky due to damage from related or unrelated trauma.

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Comparisons with St Louis encephalitis virus

- observed a range of neuroinvasive phenotypes

  neuroinvasive, attenuated, non-invasive

  [Monath *et al.* 1980; AJTMH 29:948-962]

- neuroinvasive phenotypes are linked to virus strain genotype

  [Trent *et al.*, 1981; Virology 114:319-332]

- phenotypes are conserved in mouse and hamster models

  [Monath, Cropp & Harrison, 1983; Lab Invest 48:399-410]

- similar presentation and progression of disease in animals

Neuroinvasion is via the olfactory nerve for SLE virus (and MVE virus?)

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Animal hosts

- Bird
- Horse
- Human
- Hamster
- Mouse

Birds

- Primary vertebrate host of WN virus.
- Act as amplifying host; high viremias.
- Pathology: Meningoencephalitis and mycarditis
- Viral load in brain, kidney, and heart.

Horses

- Polioencephalomyelitis type-disease with multifocal lesions.

Humans

- Fatal cases have encephalitis or meningoencephalitis involving brainstem and spinal cord.

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Hamster model

- Xiao et al. EID 7, 714-721, 2001
- Used intraperitoneal route of inoculation.
- Histopathologic changes first in brain, followed by spinal cord.
- Direct virus infection responsible for neuronal damage.
- Focal distribution of viral antigen.
- Virus not found in olfactory bulbs → virus enters brain by crossing blood-brain barrier?

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Mouse

- Highly neurovirulent and neuroinvasive.
- Neuroinvasion not via olfactory route.
- Neuroinvasion different to SLE virus.

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WN virus strain virulence comparisons

19 strains of WN virus (inc. 2 Kunjin)

- sequence 3' non-coding region for phylogenetic analysis
- i.p. LD_{50} in 3-4 wk female NIH Swiss mice
- i.c. LD_{50} in 3-4 wk female NIH Swiss mice (selected strains)
- i.p. inoculation in 3-4 wk female Golden Syrian hamsters (selected strains)
- i.p. LD_{50} in 3-4, 7-8 and 15-16 wk female NIH Swiss mice (NY99 strain 385-99 [USA99b] only)
- i.n. LD_{50} in 3-4 wk female NIH Swiss mice (selected strains)

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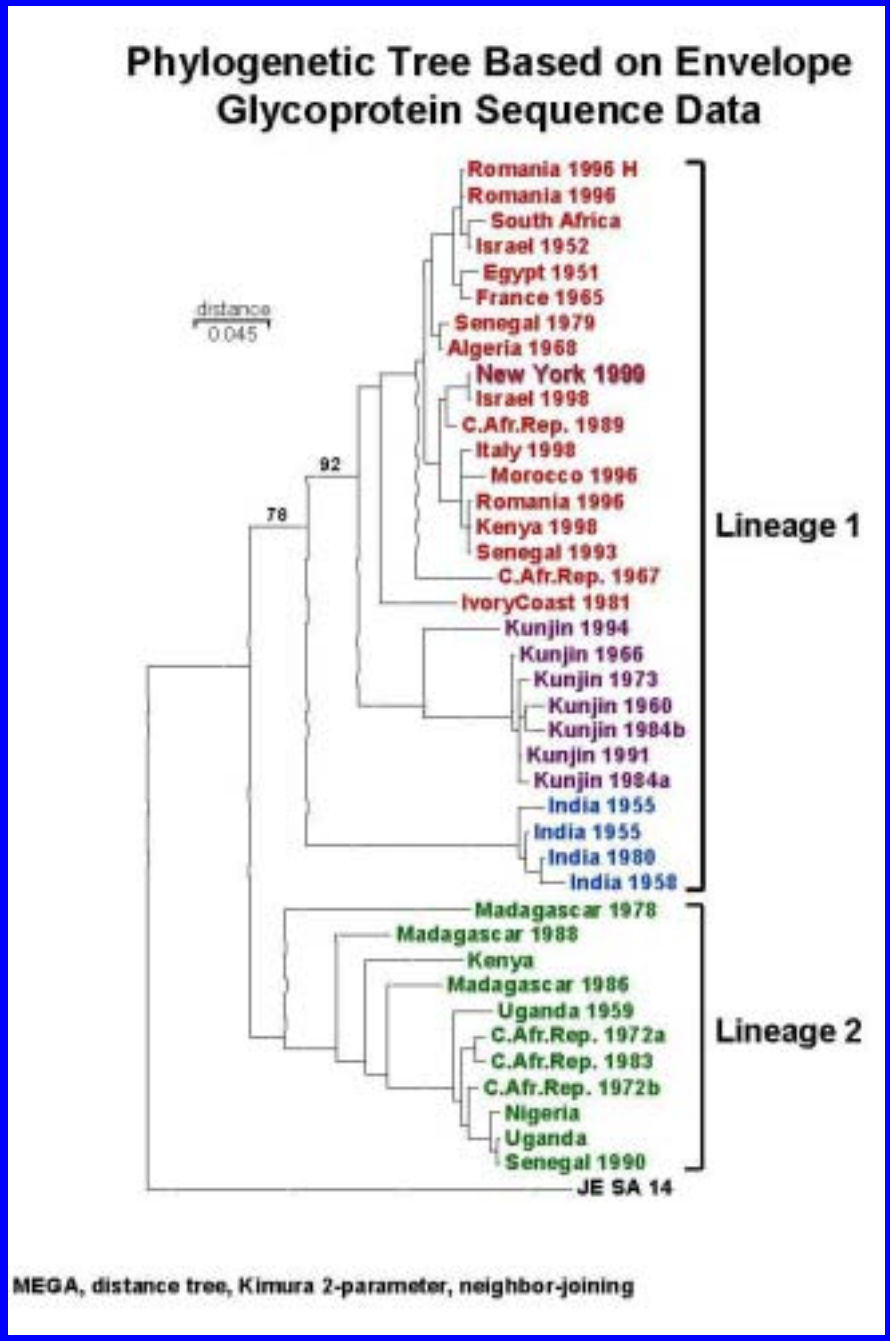

Lanciotti et al. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern U.S. [Science 286:2333-337.]

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Designation	Strain	Year	Group
CAR67	ArB-310/67	1967	I
NIG65	IbAn7019	1965	I
SEN79	ArD-27875	1979	I
USA99a	31A	1999	I
USA99b	385-99	1999	I
IND68	68856	1968	I
EGY50	Egypt101	1950	I
ETH	EthAn4766	1971	I
AUS60	MRM16 (Kunjin)	1960	KUNJIN
AUS91	K6453 (Kunjin)	1991	KUNJIN
IND57	IG-15578	1957	INDIA
IND80	804994	1980	INDIA
SEN90	ArD-76104	1990	II
CAR82	ArB3573/82	1982	II
SA58	SAH-442	1958	II
SA89	SPU116-89	1989	II
MAD88	ArMg-979	1988	II
CYP68	Q3574-5	1968	II

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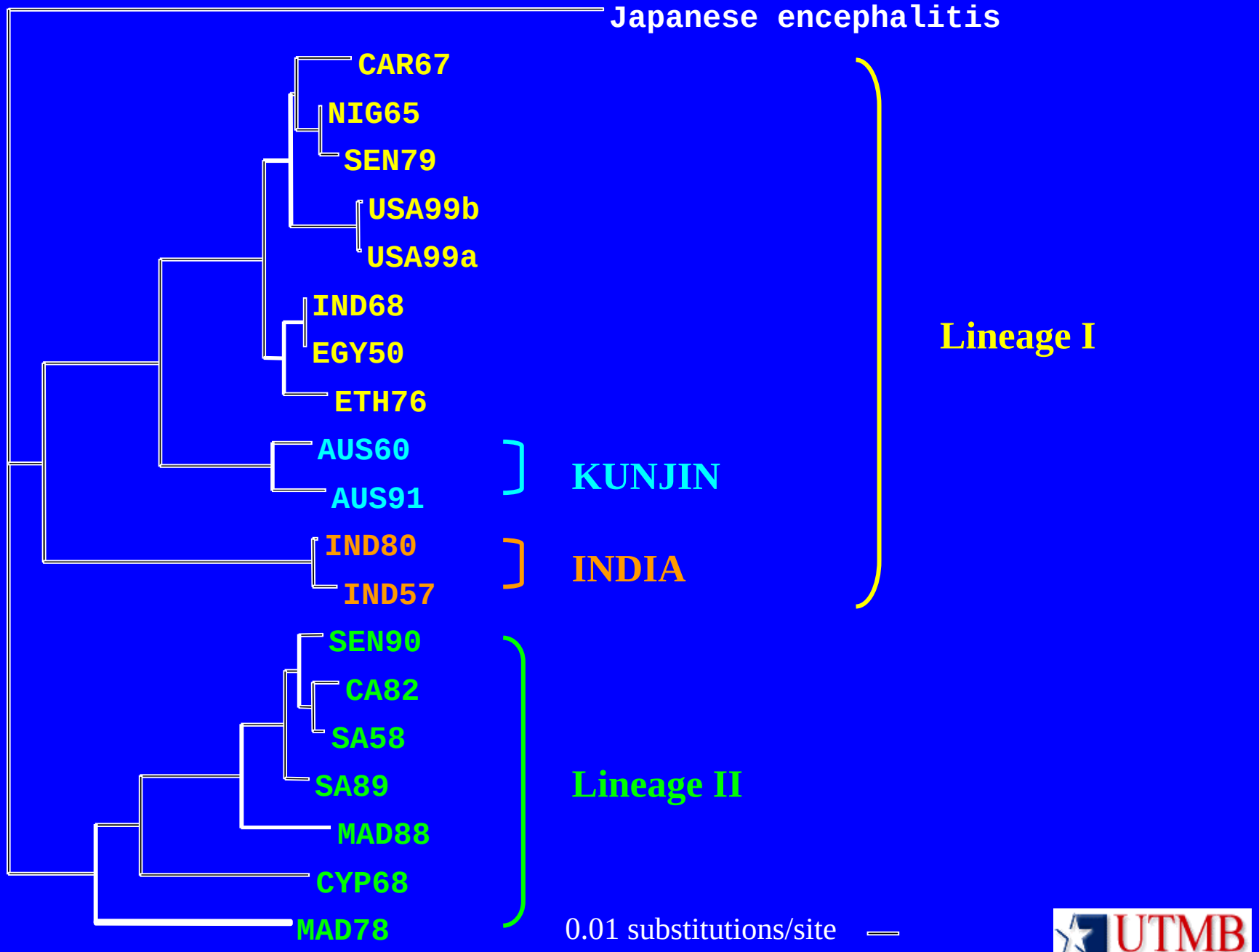
Japanese encephalitis
CAR67
NIG65
SEN79
USA99b
USA99a
IND68
EGY50
ETH76
AUS60
AUS91
IND80
IND57
SEN90
CA82
SA58
SA89
MAD88
CYP68
MAD78
Lineage I
KUNJIN
INDIA
Lineage II
0.01 substitutions/site

WN virus mouse neuroinvasion phenotypes

(by i.p. inoculation)

INVASIVE

- LD_{50} ranges from ~50 - <1 pfu (majority <10 pfu)

ATTENUATED

- scattered mortality over range of doses; LD_{50} not calculable

NON-INVASIVE

- no morbidity/mortality at any dose; $LD_{50} \geq 10^4$ pfu

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WN VIRUS STRAINS HAVE SIMILAR MOUSE NEUROVIRULENCE CHARACTERISTICS

(by i.c. inoculation)

Virus	Intraperitoneal inoculation		Intracerebral inoculation	
	LD_{50} (pfu)	Average survival time ± s.d. (days)†	LD_{50} (pfu)	Average survival time ± s.d. (days)†
SEN79	**0.2**	**8.0 ± 1.0**	**0.5**	**6.4 ± 0.9**
USA99b	**0.5**	**9.2 ± 2.2**	**0.1**	**6.2 ± 0.4**
EGY50	**50**	**7.7 ± 0.6**	**0.7**	**5.2 ± 0.4**
AUS91	**≥ 10,000**	**n/a**	**3.2**	**7.8 ± 1.3**
SEN90	**50**	**8.5 ± 0.7**	**1.5**	**5.4 ± 1.5**
SA58	**3.2**	**7.8 ± 0.8**	**0.3**	**7.0 ± 0.0**
SA89	**5**	**8.8 ± 1.9**	**0.3**	**6.2 ± 0.4**
CYP68	**>10,000**	**n/a**	**0.5**	**5.2 ± 2.7**

† for 1000 pfu dose of virus

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Intranasal inoculation of WN virus strains

Virus	SMB passage	i.c. LD_{50} (pfu)	i.n. LD_{50} (pfu)	i.p. LD_{50} (pfu)
USA99b	0	0.1	200	0.5
CYP68	2	0.3	1250	>10,000
SA58	4	0.3	200	3.2
CAR67	10	Not done	5000	12.6
EGY50	13	0.7	500	50

SMB = suckling mouse brain

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Neuroinvasive phenotype of WN virus strains is conserved in a hamster model

Strain	# surviving (out of 5)	A.S.T. ± s.d.
USA99b	0	8.8 ± 0.8
SEN79	0	9.2 ± 0.4
SA58	0	8.2 ± 1.1
IND80	4	12
CYP68	5	n/a
MAD78	5	n/a

Hamsters inoculated i.p. with 10^4 pfu of selected WN virus strains.

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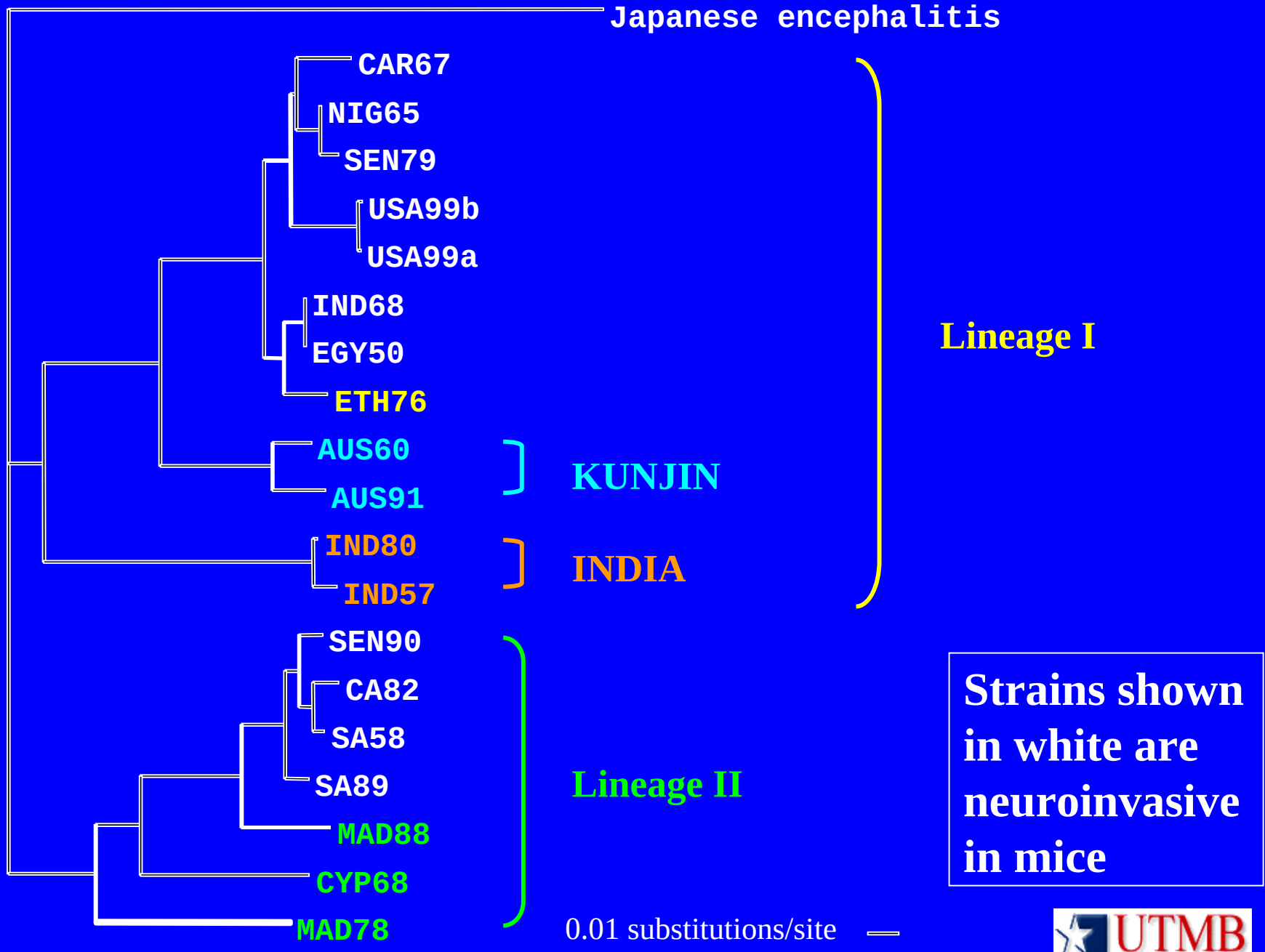
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IND57
SEN90
CA82
SA58
SA89
MAD88
CYP68
MAD78
Lineage I
KUNJIN
INDIA
Lineage II
Strains shown in white are neuroinvasive in mice
0.01 substitutions/site
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Conclusions of mouse virulence studies

1. WN virus strains differ in neuroinvasive phenotype in mouse and hamster models.
2. Neuroinvasive phenotype is associated with particular subtypes within lineage I and II.
3. Mouse virulence of neuroinvasive WN virus strains is high compared to other mosquito-borne flaviviruses
    - closeness of i.p. and i.c. LD_{50} values
    - lack of age-related resistance to infection in mice (USA99b)
4. Lack of i.n. infectivity suggests the mechanism of neuroinvasion is probably via movement across the blood-brain barrier.

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Flavivirus Genome

- ss (+) RNA genome → mRNA
- Approximately 11 kb
- 5'-m^7GpppAmp cap
- Lacks 3'-polyA tail
- Codes for
  - 3 structural proteins
    - Capsid (C), membrane (prM/M), envelope (E)
  - 7 non-structural proteins
    - NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5

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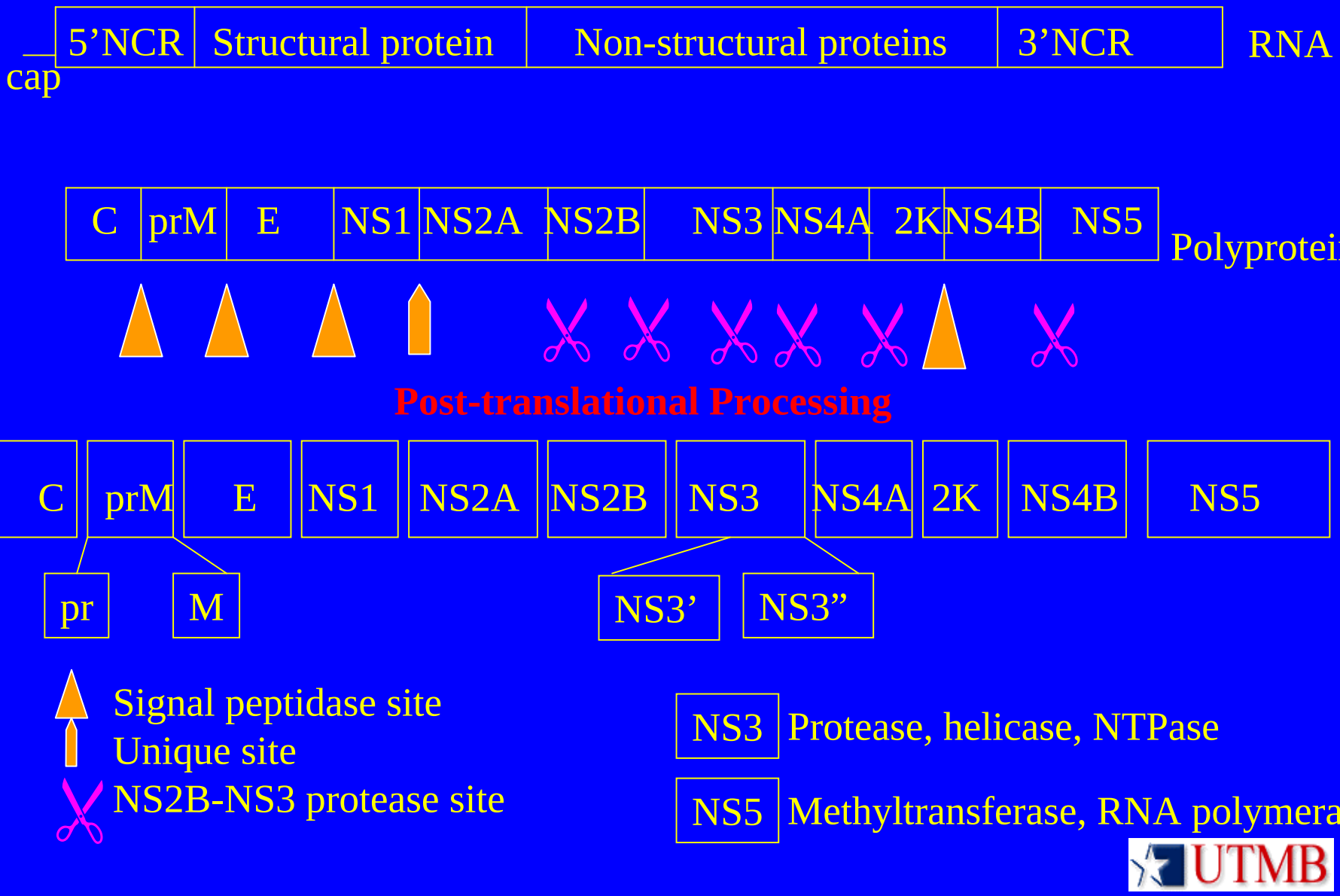
cap
5’NCR
Structural protein
Non-structural proteins
3’NCR
RNA
C
prM
E
NS1
NS2A
NS2B
NS3
NS4A
2K
NS4B
NS5
Polyprotein
Post-translational Processing
C
prM
E
NS1
NS2A
NS2B
NS3
NS4A
2K
NS4B
NS5
pr
M
NS3’
NS3”
Signal peptidase site
Unique site
NS2B-NS3 protease site
NS3
Protease, helicase, NTPase
NS5
Methyltransferase, RNA polymerase

Attenuating Mutations

- Envelope protein.
- Deletions in the Capsid protein of tick-borne encephalitis virus.
- Deletions in the 3’ untranslated region of dengue-1,-2 and -4, West Nile and Langat viruses.
- Nonstructural proteins??

E-protein

- Approximately 54 kDa
- Dimer positioned parallel to virus surface
- Three domains
  - I- Central domain
  - II- Dimerization domain
  - III- Immunogenic/Receptor binding domain
    - 10.5 kDa
    - Single disulfide bridge

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Variable residues in domain III of WN virus strains

Residue	USA99b	Kunjin	SA58
E310	Lys	Arg	Lys
E312	Leu	Leu	Ala
E332	Thr	Thr	Lys
E338	Val	Ile	Val
E365	Ala	Ser	Ala
E369	Ala	Ala	Ser

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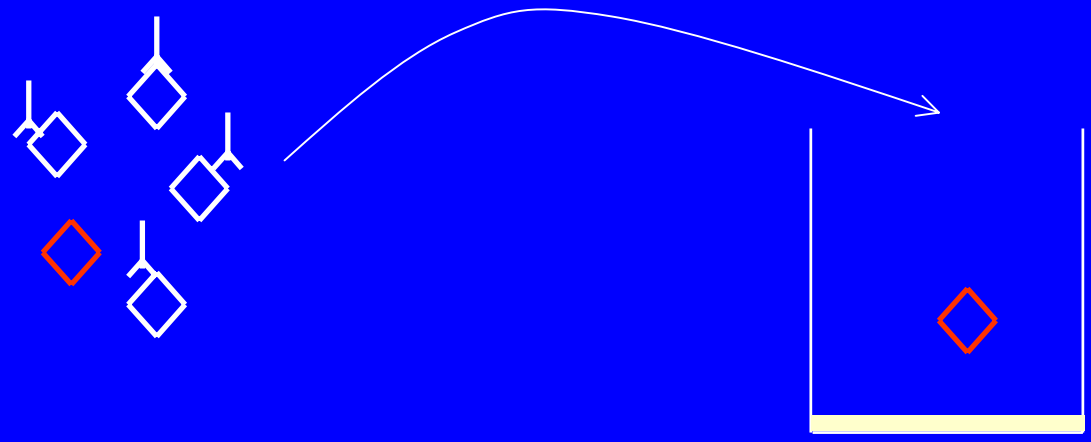
Neutralization escape variants

Variability in virus populations allows the selection of escape variants.



virus
+
neutralizing
monoclonal antibody

MAb^R virus

Membrane receptor preparation binding assays

Another potential measure of variations in WN virus virulence??

Previous MRP binding studies:

Japanese encephalitis virus and mouse brain MRPs:

- selected MRP binding escape variants with reduced virulence

Yellow fever virus and monkey brain or liver MRPs:

- observed differences in binding of neurotropic and viscerotropic strains
- selected variants with attenuated mouse neurovirulence

Langat virus and mouse or human brain MRPs:

- selected variants with reduced mouse neurovirulence

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WN virus strain MRP binding characteristics

Virus	i.p. LD_{50} (pfu)	Mouse brain MRP binding index*
CAR67	12.6	1.2
NIG65	3.2	1.2
SEN79	0.2	2.1
USA99a	0.5	1.0
USA99b	0.5	1.0
IND68	3.2	>3.8
EGY50	50	2.2
AUS60	≥ 10,000	1.2
AUS91	≥ 10,000	0.2
IND57	n/a	2.4
IND80	n/a	1.3
SEN90	50	1.2
CAR82	0.8	1.0
SA58	3.2	3.3
SA89	5.0	1.2
MAD88	n/a	2.7
CYP68	n/a	3.5
MAD78	≥ 10,000	0.9

n/a – LD_{50} could not be calculated reliably

* Binding index is $\log_{10}$ reduction in virus titer following incubation with MRP

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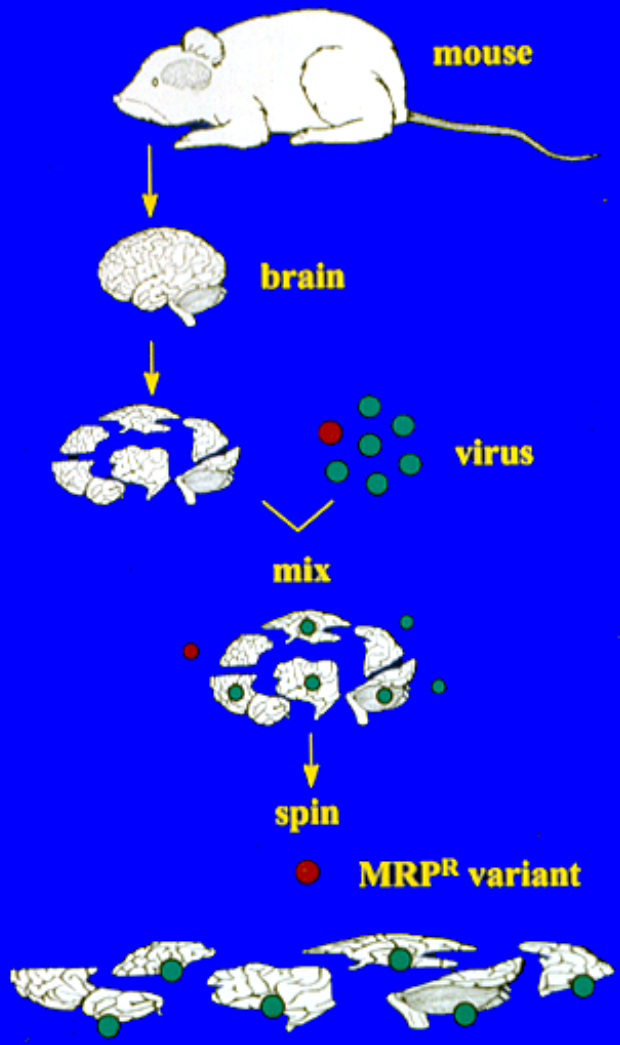
MRP binding assays and isolation of MRP variants



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