Japanese Encephalitis

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Scientific Staff

Dr. MM Gore	Scientist F	goremm@icmr.org.in
Dr. VP Bondre	Scientist C	vpbondre@yahoo.com
Mr. GN Sapkal	Scientist B	gajanan_pune@yahoo.com

Technical Staff

Mrs. Sulabha Athawale	Senior Technical Officer
Mrs. AV Shendrikar	Senior Technical Assistant
Mr. VM Ayachit	Research Assistant
Dr. DP Gangale	Research Assistant
Mr. BY Kadam	Technical Assistant
Mrs. SA Mahamuni	Technician
Mrs. V. Sankararaman	Technician

Research Fellows

Mrs. Reshma Kulkarni
Ms. Monaro Biswas
Mr. M. Feraz Ahesan
Mr. Roopesh Gangwar
Ms. S. Harini

Project Fellows

Mr. Pradip Fulmali

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Investigation of an outbreak of encephalitis in Gorakhpur, UP

MM Gore, VP Bondre, GN Sapkal, PV Fulmali, V Shankaraman, VM Ayachit and D. Gangale.

NIV team deputed at Gorakhpur during August - September 2007 has investigated acute encephalitis syndrome (AES) cases for viral etiology. According to State Government Health Services, 2098 cases were admitted from January December 2007 in the region and 398 deaths (CFR 18.97%) were reported. A total number of 1277 clinical specimens which includes CSF (606), acute sera (601), rectal swabs (32), throat swabs (32) and brain biopsies (05) were collected. All the clinical specimens were tested for detection of etiological agent by various tests.

Investigation of specimens for Japanese encephalitis virus infection.

A total of 663 sera were tested for presence of anti JEV IgM antibodies. 63/663 (9.5%) CSF's and 70/646 (10.8%) sera were tested positive for anti JEV IgM antibodies. Most of these were serum-CSF pairs. Presence of JE virus genome in CSF and serum was tested by JEV specific RT-PCR and real time RT-PCR assays. 46/488 (9.4%) CSF's were tested positive for JE virus genome. 3/488 CSF's tested positive for both the anti JEV IgM antibodies and JEV specific real time RT-PCR. Thus total positivity for JEV etiology was about 18%. In addition 141/ 602 (23.42%) sera were tested positive for anti JEV antibodies by CPE neutralization assay.

Isolation of JE virus was attempted by inoculating CSF's in infant mice. Out of 76 CSF samples inoculated 23 suspected mouse brains were tested for presence of JEV by RT-PCR. Two isolates of JE virus have been obtained from 2007 outbreak. Sequence analysis of partial C - PrM region indicates close sequence similarities with GP-78 isolated from 1978 Gorakhpur epidemic.

Testing of specimens for enterovirus diagnosis

Along with JEV diagnosis, specimens were also tested for enterovirus diagnosis by RT-PCR. 10 / 488 CSFs tested positive by enterovirus specific generic RT-PCR. Sequence analysis indicated close similarities with EV76 (4), Echo 11 (3), EV84 (1), Cox 16 (1) and Echo 30 (1). In addition, EV 76/89 was detected in 2 /11 CSF specimens obtained during the month of March 2007.

Isolation of the viral agent from patient's CSF

Enterovirus isolation was attempted by inoculating CSF specimens in different cell lines. A total of 319 CSF's were inoculated in Vero E6, Rhabdo myosarcoma (RD) and BHK cell lines. 30 cultures showed some changes in the cell morphology after 2-3 passages. Out of these, two passage - 2 cultures showed EV like particles by electron microscopic analysis and EV was detected in 3 cultures by RT-PCR (2- EV 76 & Echo 11). Additionally JEV was detected by RT-PCR in 4 cultures. A set of CSFs were also tested for diagnosis of encephalitis associated RNA viruses including JE, Entero, Alpha, Nipah, Hendra by RT-PCR and DNA viruses including HSV and VZV by specific PCR assays.

Molecular characterization of newly identified viruses

Background

Complete genome sequence of newly identified Enterovirus 76 isolated from human clinical specimen collected during 2006 Gorakhpur encephalitis outbreak was obtained analyzed. Similarly complete genome sequence of

newly identified Bagaza virus isolated from *Culex tritaenorhynchus* mosquito pool collected during encephalitis outbreak in Kerala during 1996 was obtained and analyzed. Additionally complete genome sequence of highly pathogenic West Nile virus strain 68856 isolated from *Rousettus leschenaultia* (fruit bat) from Karnataka during 1968 was obtained and analyzed.

Molecular characterization of Enterovirus 76 isolated from 2006 Gorakhpur encephalitis outbreak

VP Bondre, GN Sapkal, PV Fulmali, V Shankaraman, VM Ayachit and MM Gore.

Background

Partial genome sequences obtained by EV specific RT-PCR from human clinical specimens and typing of the virus by partial VP1 sequence using tissues culture amplified viruses showed maximum similarities with human Enterovirus 76 first time documented in Bangladesh from acute flaccid paralysis cases during 1999-20001. Complete genome sequence of one of the Indian isolate was obtained and analyzed.

Objective

- Full length genome sequencing of Enterovirus 76 and data analysis.

Work done

Two tissue culture grown viruses (specimens 0623407 & 0623350) were processed for the genotyping among Enteroviruses by RT-PCR amplification of 890 nt product from VP1 / 2A region (2439-3329). The sequence analysis confirmed both the isolates as Human Enterovirus -76. Phylogenetic and nucleotide identity analysis indicate closer relationship with Human Enterovirus 76 isolated in Bangladesh during 1999-2000 **(Fig 1)**.

Primers for complete genomic sequencing of EV-76 isolate were designed by aligning all the available full genome sequences from GenBank. A 7400 nt sequence of isolate 0623407 was obtained from both the ends and analyzed. The sequence was compared with worldwide isolated Enteroviruses and its phylogenetic relationship was determined.

Findings

Complete genome sequence analysis of human EV isolated from GKP 2006 encephalitis outbreak revealed differences from most of the worldwide isolated Enterovirus strains. However, the Indian EV strain showed maximum sequence similarity of 91-92% with Enterovirus 76 isolated from Bangladesh during 1999 -2001 from acute flaccid paralysis cases.

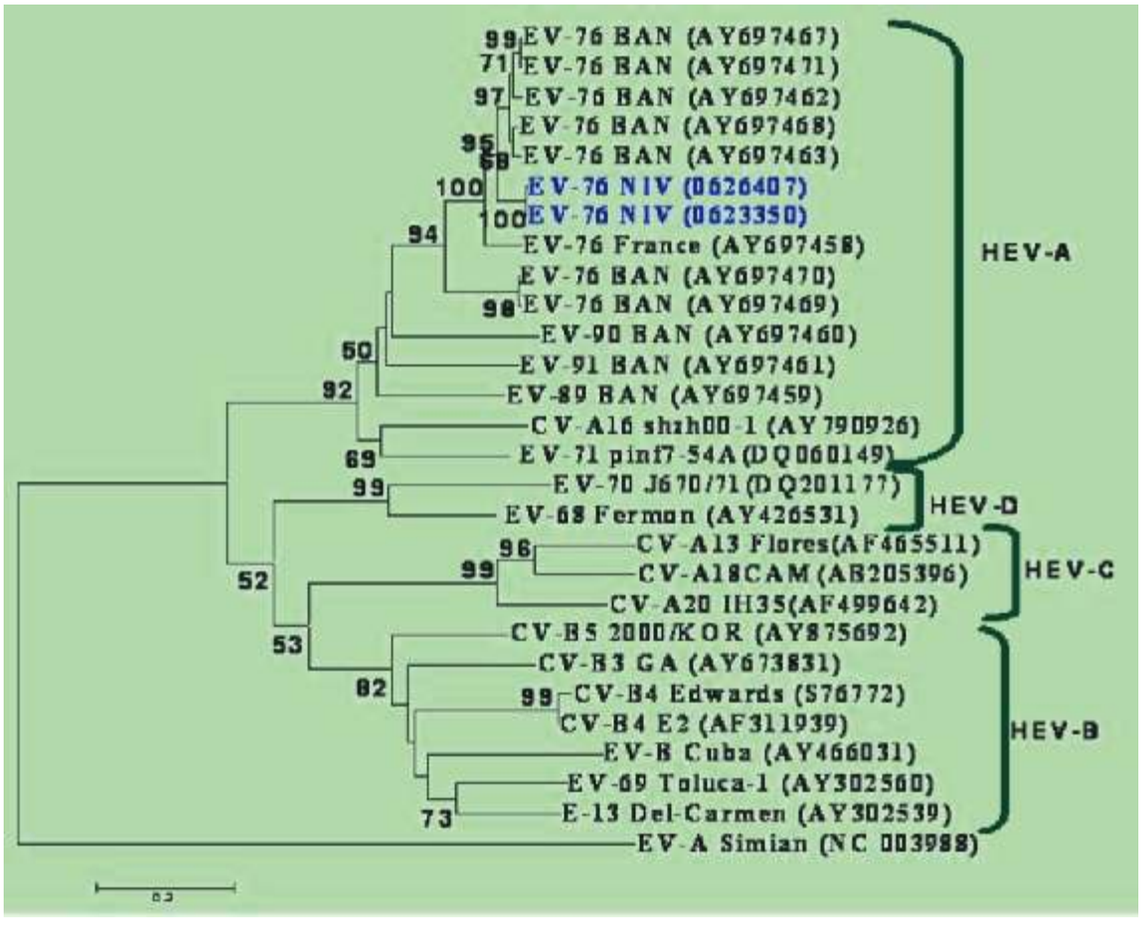


Fig. 1. Phylogenetic analysis of human enterovirus using 930 nt VP1/2A gene sequence.

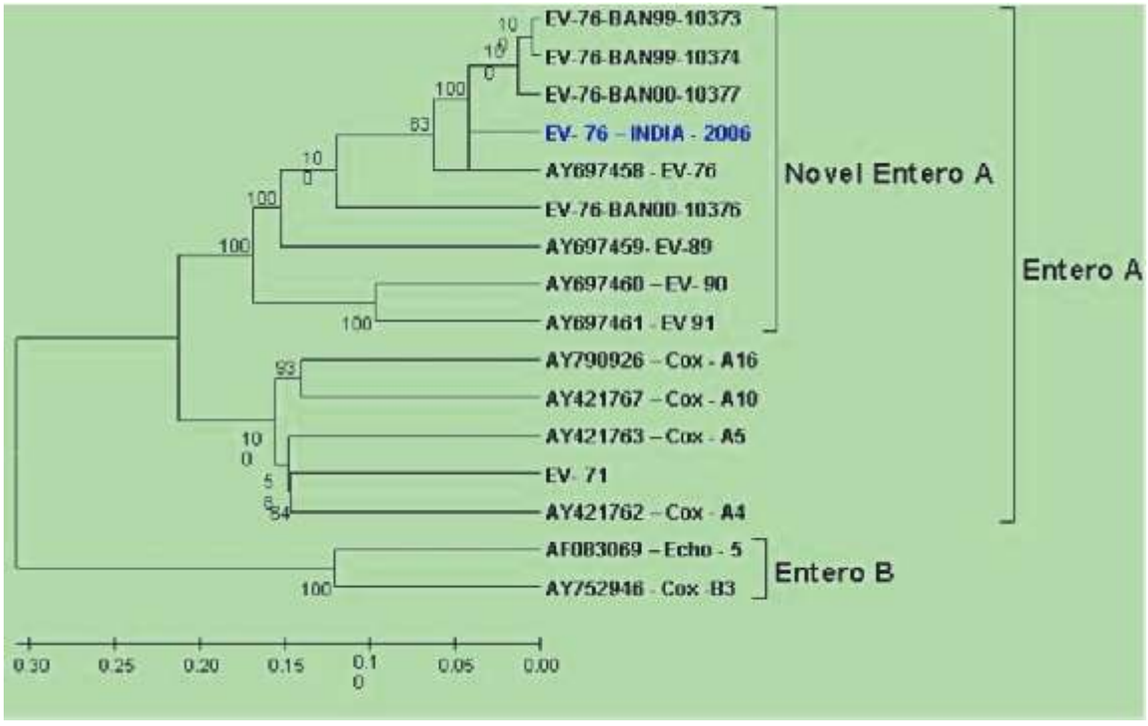


Fig.2. Phylogenetic analysis of human enterovirus 76 using complete genome sequence.

Genomic characterization of Bagaza (BAG) virus isolated from *Culex tritaenorhynchus* mosquitoes collected from Kerala during encephalitis outbreak

VP Bondre, GN Sapkal, Y. Prasanna, PV Fulmali, V. Shankaraman, D. Gangale, VM Ayachit, G. Jacob and MM Gore.

Background

An arbovirus was isolated from a pool of female *Culex tritaenorhynchus* mosquitoes collected from Punna Kunnam region of Allappuzha district of Kerala during an encephalitis outbreak in 1996. Preliminary serological tests including complement fixation and neutralization tests using hyper immune serum against JEV, WNV, CHPV, CHIKV, Sindbis, Bital and DENV 1, 2, 3, 4 characterized it as JEV and WNV cross reactive virus. Studies were carried out to determine the genetic and biological relationship of the arbovirus isolate with JE, WN and other flaviviruses.

Objective

- Molecular characterization of an arbovirus isolated from mosquitoes collected during an encephalitis outbreak in Kerala in 1996.

Work done

The evidence on the arbovirus isolate as Bagaza virus came from RT-PCR amplification and sequence analysis of NS5 region amplified using Flavivirus specific universal primers. 95% sequence similarities with Bagaza virus followed by 93% similarities with Israel turkey meningoencephalitis virus (ITV) suggests genetic relatedness of the isolate with Ntaya group of flaviviridae. Complete genome sequence of the newly characterized Bagaza virus was obtained by RT-PCR amplification and sequencing of genome using primers designed by aligning all the full length genome sequence of related viruses. The 10281 nt full genome sequence of Indian Bagaza virus (GenBank accession number EU684972) represents complete open reading frame (ORF) coding 3426 amino acids. Complete genome sequence analysis showed close similarities with Ntaya group of viruses with maximum similarity of 94.9% with African Bagaza virus - Kuno strain. However as compared to Kuno strain, Indian Bagaza virus showed 515 nucleotide changes leading to a total number of 77 amino acid alterations throughout the ORF. Additionally BAGV-Ind showed one deletion (7424) and 4 additions (7438-39, 7444 and 7463) at the C terminal region of NS4B encoding protein.

To investigate the phylogenetic relationship of Indian Bagaza virus with other members of flaviviridae, multiple nucleotide and putative amino acid alignments were made. Phylogenetic analysis based on complete ORF as well as partial nucleotide sequences and putative amino acid sequences generated close relationship of viruses transmitted by *Culex* mosquitoes, *Aedes* mosquitoes, ticks and unknown vectors. Both the African and Indian Bagaza virus isolates clustered together on phylogram constructed with complete ORF encoding sequence of various members of flaviviridae **(Fig. 3)**. Bagaza virus strains clustered together with members of *Culex* clade mostly causing encephalitis disease in man. Complete ORF sequence analysis of both Indian and African strains showed percent nucleotide identity (PNI) of 95±0.20% with each other. Partial envelope and NS5 sequence based analysis also showed similar phylogenetic relationship of BAGV with other memenrs of flaviviridae as obtained with complete ORF sequence analysis. Other members of Ntaya group including Tembusu virus, Ntaya virus and ITV also clustered with BAGV in phylograms constructed using partial envelope and NS5 sequences. Envelope

sequence comparison of BAGV-Ind with BAGV-Kuno (AF372407) and Dak- Ar-B209 (AF372407) strains showed PNI of 95.12±0.76 and 95.54±0.79% respectively while BAGV-Kuno showed PNI of 99.67±0.25% with Dak- Ar-B209. With partial NS5 sequence, BAGV-Ind showed PNI of 99.90±0.10% with BAGV-Kuno (AY632545) and Dak-Ar-B209 (AF013363) while 99.90±0.10% PNI was observed between BAGV-Kuno and Dak- Ar-B209.

Findings

The partial as well as complete genome sequence analysis of the arbovirus classified as Bagaza virus which is a member of Ntaya group of flaviviridae transmitted by *Culex* mosquitoes and are mostly characterized as encephalitic viruses.

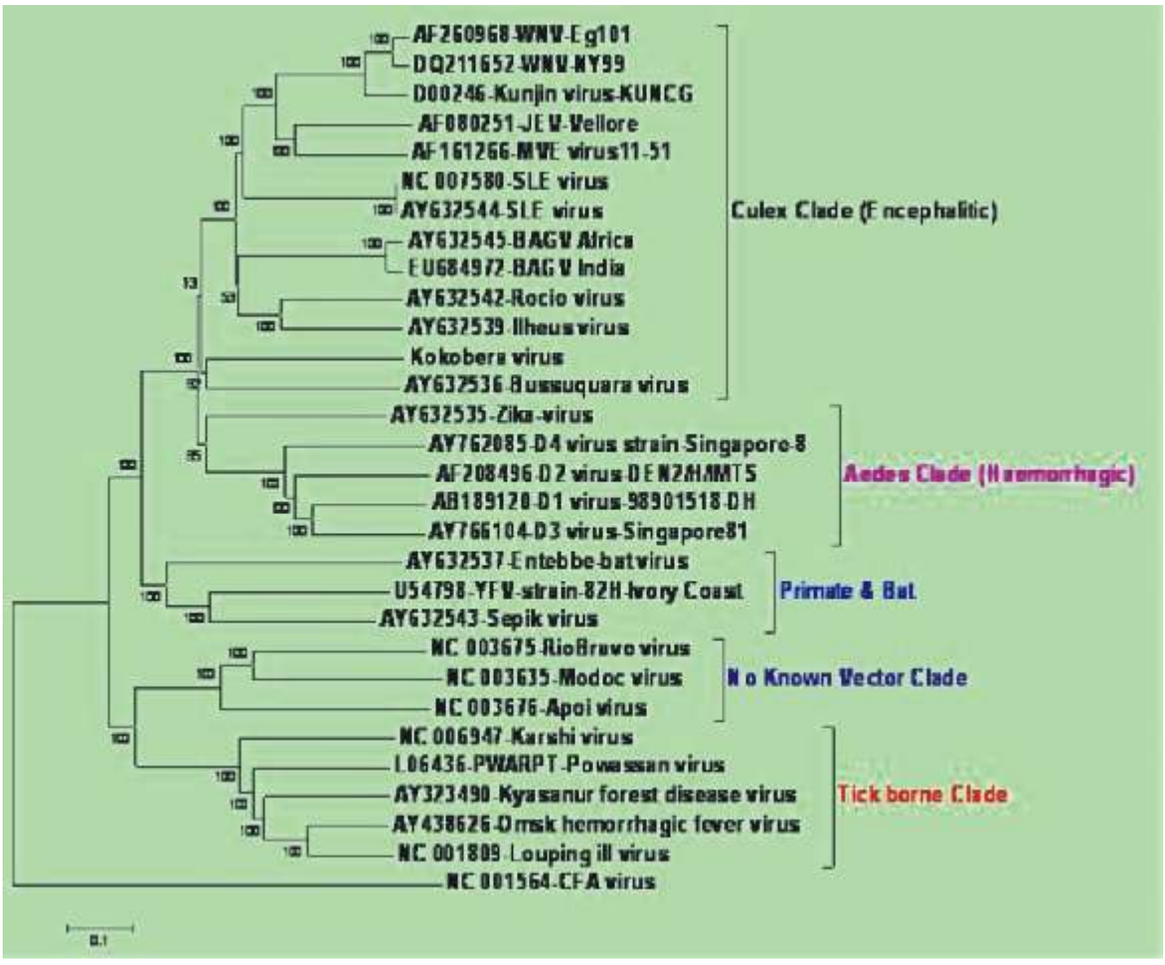


Fig. 3. Phylogenetic analysis of Bagaza virus using complete genome sequence.

Full genomic characterization of a highly virulent, lineage 1 West Nile virus (68856) strain isolated from bat

VP Bondre, GN Sapkal, V. Shankaraman, PV Fulmali, D. Gangale, VM Ayachit, and MM Gore.

Background

West Nile virus strain 68856 isolated from bat in 1968 is neurovirulent and peripherally pathogenic in mice. Several studies using 68856 characterized it as highly virulent strain as compared to other WN strains isolated in India. Comparative biologically characteristics are shown to be distinct from Egyptian prototype strain Eg-101. We have documented difference in cross neutralization assay of 68856 and Eg-101. Our genetic studies based on partial genomic sequence in C-prM-E region indicate that the strain is genetically distinct from other Indian strains and sequence similarity with Eg-101 was observed. However, genetic differences with Eg-101 are reported in NS1 region by researchers in other laboratories.

Work done

In addition to the difference in cross neutralization studies, difference in cross protection through immune lymphocyte was studied using immune cell transfer studies. In order to understand the sequence changes associated with altered biological properties of Eg-101 and 68856, we obtained complete genomic sequence of 68856. The sequence was compared with all sequences of globally reported human pathogenic and moderately pathogenic strains available in GenBank. The sequence showed 96% similarity with most of the lineage 1 epidemic strains isolated from humans in Western hemisphere. However complete genomic sequence of 68856 showed closer relationship with Eg-101 (Egypt) with 99% and Chin -01 strains with 97% similarities. As compared to Eg-101, altogether difference in eight nucleotides 2 in 5' NTR, 4 in Env, 1 in NS2A & 1 in NS5 coding regions was recorded that lead to difference in 5 amino acids 4 in Env and 1 in NS2A protein coding regions. As compared to Eg-101 complete amino acid coding ORF, the 68856 showed 4 amino acid changes in envelope region at P156-S, T332-K, T366-A, H398-Y and one change at V138-A in NS2A region. Among Flaviviruses, amino acids 154-156 fall in NYS motif involved in receptor and antibody binding. The altered residues in the receptor-binding domain determine virulence of the strains. Alteration of S156 to F or P or any other amino acid residue results in neurovirulence property of the strain. Studies replacing S156 to other amino acids by site directed mutagenesis altered the virulence in animal experimentations. Role of other amino acid changes T-K, T-A, H-Y and V-A in determining the biological properties of WN virus are not yet clear.

Findings

Complete genome sequence analysis of West Nile virus strain 68856 shows 26% genetic divergence with Indian WNV isolates and close similarity with lineage 1 pathogenic strains. Detailed studies are necessary to determine the role of P-S alteration in virulence of the virus.

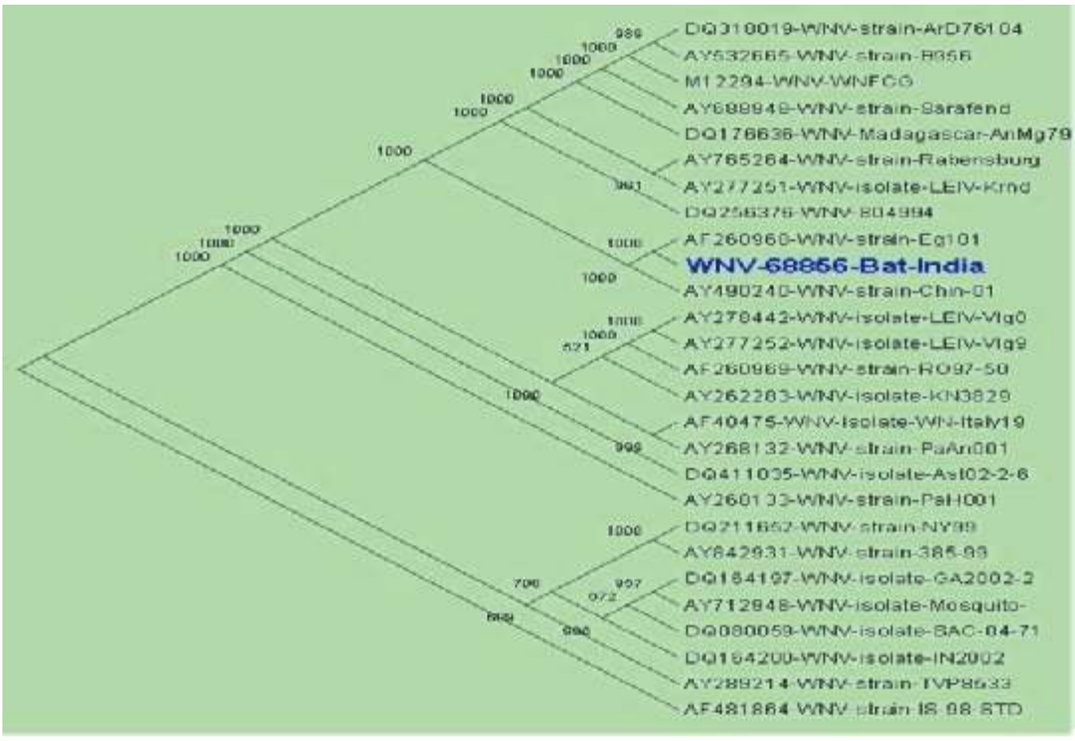


Fig.4. Phylogenetic analysis of WNV 68856 strain using full genome sequence.

Epidemiology and immune response against Japanese encephalitis virus strains at molecular level in North-Eastern regions of India

MM Gore, VP Bondre, SA Khan, P. Dutta, GN Sapkal, PV Fulmali, D. Gangale, V. Shakaraman, VM Ayachit and RS Gangwar

Virus isolation and detection of JE and WN virus from patient's CSF

332 Cerebrospinal fluid and 200 sera were collected from suspected encephalitis patient during May-Aug 2007 in northeastern region. The CSF's were processed for virus isolation by inoculation in infant mice and susceptible cell lines. Brains harvested from suspected sick mice were tested for the detection of JE and WN viruses by virus specific single step RT-PCR. Five isolates characterized as 3 JE virus and 2 WN virus have been established in infant mouse brain. The JE and WN virus isolates are being genetically characterized by sequence analysis. Limited sequences analysis indicate closer relationship of JE virus isolates with Indian prototype strain P20778 and that of WN virus isolates with Indian isolate 804994. Further studies are in progress.

Diagnostic RT PCR of CSF's collected in the acute phase of encephalitis for JE and WN virus

Viral RNA isolated from all the 332 CSF's was processed for diagnosis of JE and WN virus infections by JE and WN virus specific RT-PCR followed by semi-nested PCR. 46/332 (13.85%) CSF's were positive for JE virus specific PCR and 9/332 (2.7%) CSF's were positive for WN virus specific PCR. All the PCR products amplified directly from CSF were sequenced and analyzed. Phylogenetic analysis based on 250 nt product amplified by semi-nested PCR directly from CSF indicate genetic similarities with most of the circulating Indian strains **(Fig. 5)**. However, three JEV sequences obtained directly from CSF were genetically distinct from strains circulating in India. Since this data is based on small genomic fragment which has to be supplemented with additional data by amplifying other larger genomic regions of the virus directly from CSF. Further studies on isolation and genetic characterization are in progress.

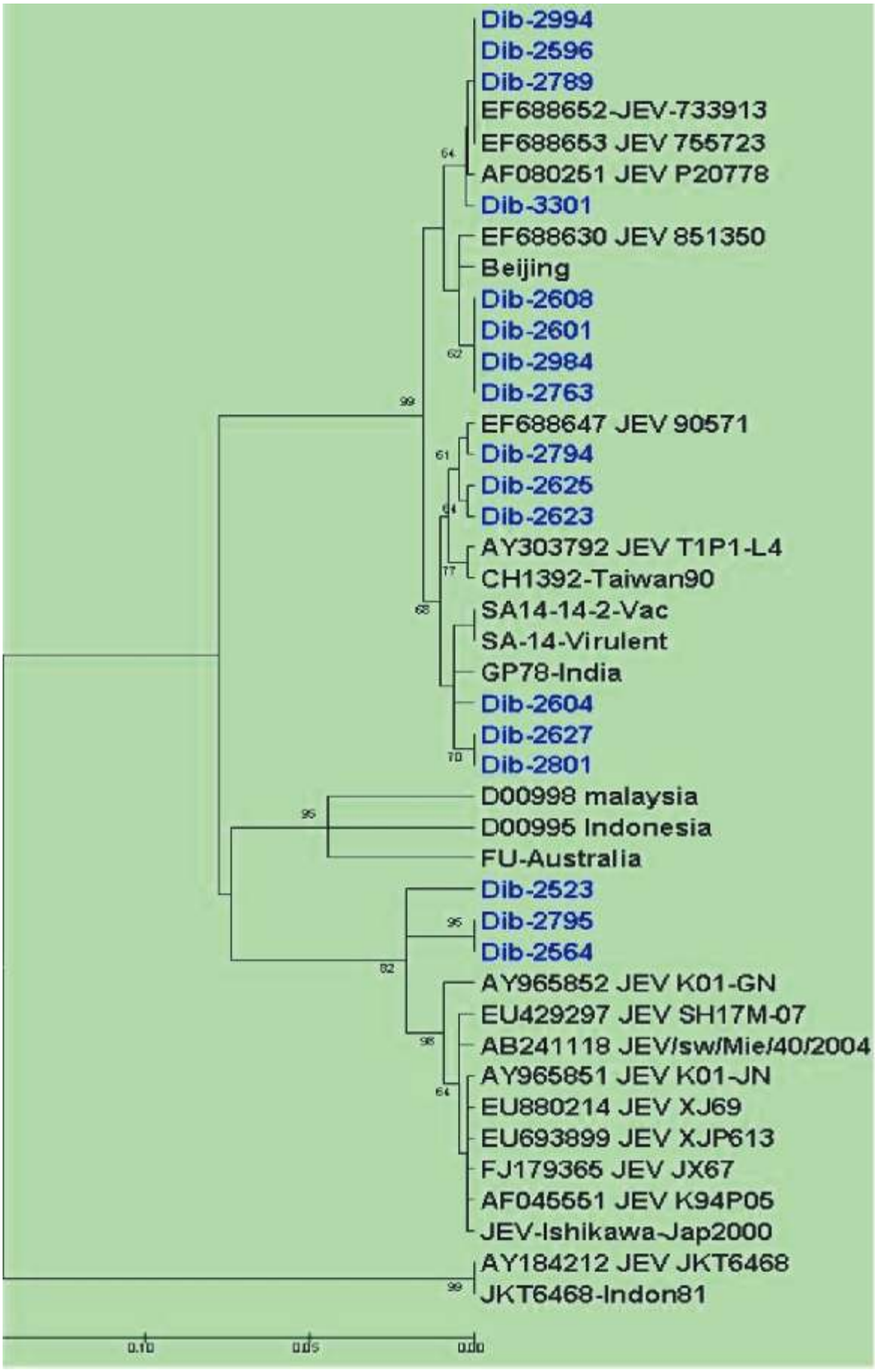


Fig.5. Phylogenetic analysis of JE sequences directly amplified from patients

Detection of anti WN virus specific IgM antibodies

The 332 CSF specimens and 200 sera were tested for detection of anti JE virus specific IgM antibodies using NIV kit at RMRC Dibrugadh. 245 / 434 CSF (56%) positive and 191 / 593 sera (32.2%) were positive for JE IgM antibodies. Additionally sera were tested for WN virus specific IgM antibodies detection using West Nile virus specific PAN-BIO Kit. 6/123 (4.87%) sera tested positive for anti WN virus specific IgM antibodies. Further serological investigations of CSF and sera are in progress.

Prevalence of JEV and WNV specific neutralizing antibodies among children in Dibrugarh.

Sera collected from children from Dibrugarh area during the year 2006 were analyzed for estimation of neutralizing antibodies against JE (733913) and WN (68856) by CPE inhibition assay. 55.87% of children (1002 sera tested) showed neutralizing antibodies against JE while 43.5% sera showed WNV specific neutralizing antibodies. 36.42% of the children did not show neutralizing antibodies against these viruses. Prevalence of JEV and WNV specific neutralizing antibodies was documented in 20.05% and 7.68% of the sera respectively. The data warns necessity of further studies to understand circulation of these viruses, their distribution and genetic relationship with other strains documented in country.

Table 1 . JEV and WNV neutralizing antibody titers among children sera from Dibrugarh.

<table>
<tr><th colspan="2" rowspan="2">Category</th><th rowspan="2">Ab Titer</th><th colspan="2" rowspan="2">Number of Samples</th><th colspan="2">% positivity within the</th></tr>
<tr><th>Sample gr</th><th>Total gr</th></tr>
<tr><td rowspan="2">JE alone</td><td>High Titer</td><td>>40</td><td rowspan="2">201</td><td>122</td><td rowspan="2">20.05%</td><td>60.69%</td></tr>
<tr><td>Low Titer</td><td>20-40</td><td>79</td><td>39%</td></tr>
<tr><td rowspan="2">WN alone</td><td>High Titer</td><td>>40</td><td rowspan="2">77</td><td>58</td><td rowspan="2">7.68 %</td><td>75%</td></tr>
<tr><td>Low Titer</td><td>20-40</td><td>19</td><td>25%</td></tr>
<tr><td rowspan="4">JE and WN both</td><td>High Titer</td><td>>40</td><td rowspan="4">359</td><td>41</td><td rowspan="4">35.82%</td><td>11%</td></tr>
<tr><td>Low Titer</td><td>20-40</td><td>160</td><td>45%</td></tr>
<tr><td>High for JE & low for WN</td><td>>40 (JE) 20-40(WN)</td><td>136</td><td>38%</td></tr>
<tr><td>High for WN & low for JE</td><td>>40 (WN) 20-40(JE)</td><td>22</td><td>6%</td></tr>
<tr><td colspan="2">Total Negative</td><td><20</td><td colspan="2">365</td><td colspan="2">36.42%</td></tr>
<tr><td colspan="2">Total</td><td></td><td colspan="2">1002</td><td colspan="2"></td></tr>
</table>

Virus isolation attempts from field collected mosquitoes

Mosquitoes collected during the outbreak season were identified and 127 mosquito pools were made. 7 pools were tested positive for JEV by antigen capture ELISA. These pools were also processed for virus isolation in infant mice and RT-PCR diagnosis. No isolation was obtained up to passage 2. Similarly these mosquito pools and infected mice brains were negative by JEV specific RT-PCR.

Standardization of ELISA using synthetic peptide (B cell)

After standardization of ELISA using patient's sera with the B cell peptides from JE, WN and DEN viruses, the test was further standardized using available survey sera with known neutralizing antibody titers. The following peptides were selected for further study after primary screening, which were used for standardization of peptide ELISA. Out of 42 peptides, a set of eight peptides that showed type specific reactions were selected for ELISA against JEV using pooled sera.

JE Specific Peptides

JE Egp 40-TLDVRMINIEASQLA-54	1JE40
JE Egp 149-SENHGNYSAQVGASQA-164	3JE149
JE Egp 288-RLKMDKLALKG-298	6JE288
JE Pre M Aligned 33-PTSKGENRG-41	42JP33

WN Specific peptides

WN Egp 147-TTVESHGKIGATQAGRF-163	11WE147
WN Egp 302-SKAFKFARTPADTG-315	14WE302
WN Pre M Aligned 19-VTDVITIPTA-28	39WP19
WN Pre M Aligned 93-SLTVQTHGESTLA-105	41WP93

The survey sera (Warangal region) with predetermined neutralizing antibody titers against JE (733913) and WN (G22886) were used on these peptides for ELISA. The antigen capture ELISA was carried out with JE (733913) and WN (G22886) viruses (captured on Hx2 cross reactive monoclonal antibody) to determine the appropriate dilution and cross reactivity of these sera in the peptide ELISA.

The NT titers of the sera were as follows :

Serum samples	JE NT titer	WN NT titer
JE (733913) Positive	>1250	30-150
WN (G22886) Positive	<10	>1250

The sera having neutralizing antibodies against WNV did not cross react with JEV peptides. However, JEV positive sera showed cross - reactivity with WNV in neutralization. Hence, these sera were selected for further standardization of peptide ELISA.

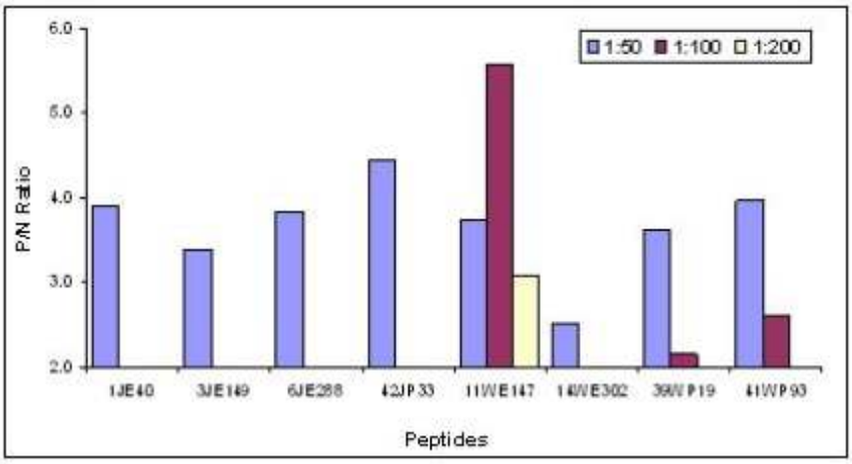


Fig. 6. Reactivity of human sera with JE and WN specific peptides in ELISA.

The peptide ELISA showed similar results with the whole virus. WNV specific peptides 11WE147 showed specific reactivity with WN Nab positive sera.

All the reagents including blocking agents (1-1.5% Gelatin, 1-2% BSA, 1-2% skimmed milk), washing solutions (0.05-0.1% T-20 with and without salt) and reaction conditions (temperature) were standardized to minimize the background noise in peptide ELISA. 1% skimmed milk as blocking agent and 0.1% T-20 as wash buffer gave optimum results in peptide ELISA whereas different salt concentrations in washing buffer did not have any effect on ELISA.

Cross-reactive epitope on JE Egp and Histone

During generation of monoclonal antibodies (MAbs) against envelope protein of JEV, two MAbs (NHA-1 and NHA-2) were obtained that were cross reactive with cellular histones. These findings were confirmed by studying the Ab3 response against anti idiotypic antibody against MAb NHA-1. Preliminary analysis suggests that the reactive epitope on JEV envelope protein might be non-sequential which is reacting with histone in western blotting. This may be due to a partial conformational and structural homology between reactive epitopes of histone and JEV envelope protein. JEV envelope protein has been demonstrated in the nucleus of infected cells, which may due to transportation in nucleus because of their structural homology. It would be interesting to understand whether the non-sequential epitopes on JEV is mimicking the sequential epitope on histone proteins. This study will lead to understand the reason of translocation JEV envelope protein to nucleus.

Determination of homology between histone and JEV envelope protein.

Amino acid alignment analysis of JEV envelope protein and hostone protein does not show simple sequence-level homology. Similarly these proteins did not have any homology between conserved regions. Similar kind of cross - reactivity between histone like protein (HLP) from *Mycobacterium tuberculosis* with these MAbs (NHA-1 and NHA-2) have been documented. A sequence similarity search using tetrapeptides from the envelope protein (after normalizing the amino acids according to their properties) gave the regions on the HLP that might be antigenic region on the histone. These have been selected for the study. The tetrapeptides sliding homology of JEV envelope protein with HLP after coding the sequences according to the above table has given the following results. On the bases of this homology following regions have been selected from HLP for this study:

HP_1: 06- LIDVLTQKLG -15

HP_2: 27- NVVDTIVRAVHKGDS -41

HP_3: 97- AEGPAVKRGVGAS -109

HP_4: 165- AKKVTKAVKKTAVKASVTKA -184

ELISA with histone peptides:

ELISA on histone peptides were carried out in nunc covalink ELISA modules. The coated peptides (Histone and JEV were also coated) were probed with NHA-1, NHA-2, HX-2 and JE polyclonal sera.

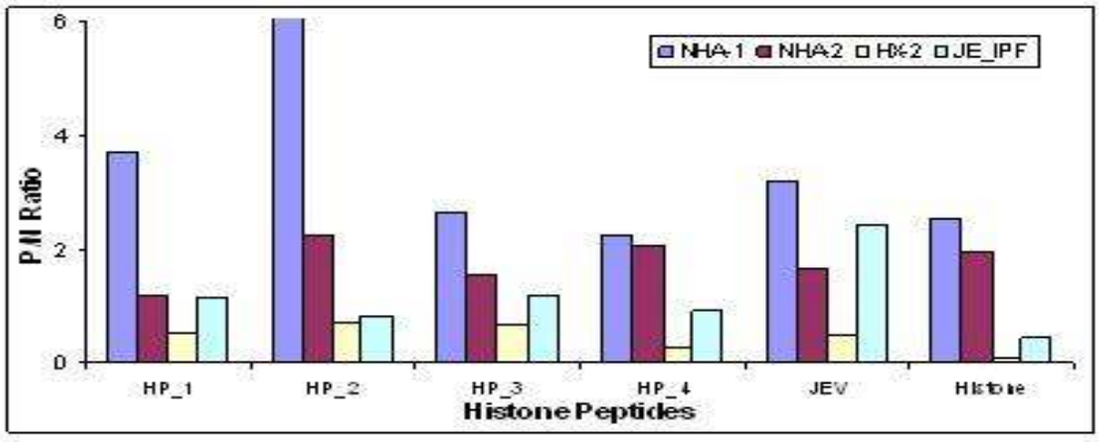


Fig. 7. ELISA on histone Peptides.

Findings

Histone peptides, histone and JEV (733913) strongly react with NHA-1 and NHA-2 monoclonal Abs. A low level of reactivity with anti - JEV polyclonal sera was observed. HP_1 and HP_2 showed higher reactivity than other peptides. No significant reactivity of HX-2 MAb was observed with any of the histone peptide or histone protein. These finding suggests that the envelope protein of JEV and histone protein might be sharing homology at structural level.

Determinants of peripheral pathogenicity of West Nile virus

GN Sapkal, VM Ayachit, VP Bondre and MM Gore.

Background

In order to determine the amino acids responsible for attenuation of West Nile virus, complete envelope proteins (E protein) coding sequence of the wild type strains WN 68856 and its neutralization escape mutants were analyzed. All these mutants showed common amino acid substitutions at position 89 Alanine- Phenylalanine, 90 Phenylalanine -Leucine, 91 Valine- Leucine. However, mutant IF1A71.1 showed two amino acid substitutions at positions 156 Serine-Proline, 242 Phenylalanine-Tryptophane which were absent in other two mutants. Of note is S156P substitution has been reported earlier in Flaviviruses and found to be involved in loss of peripheral virulence.

Objective

- To determine the altered cell tropism of mutant viruses with changes in E protein.

Work done

In our earlier work monoclonal antibodies exhibited less protection against the corresponding variants with altered pathogenicity as compared to the parent strain. Therefore, the present work was aimed to study an *In vitro* model to study the WNV infection in various cells. Two Nt escape mutants viz. NtE IF1A7 and NtE IVC3F10 along with the wild type WN 68856 viruses were used for study.

Peripheral infection of WNV encounters peritoneal exudates cells (PEC) and spleenocytes as a primary multiplication site that determines the outcome. Thus, growth of these mutants in PEC,adherent and non-adherent population of splenocytes was studied.

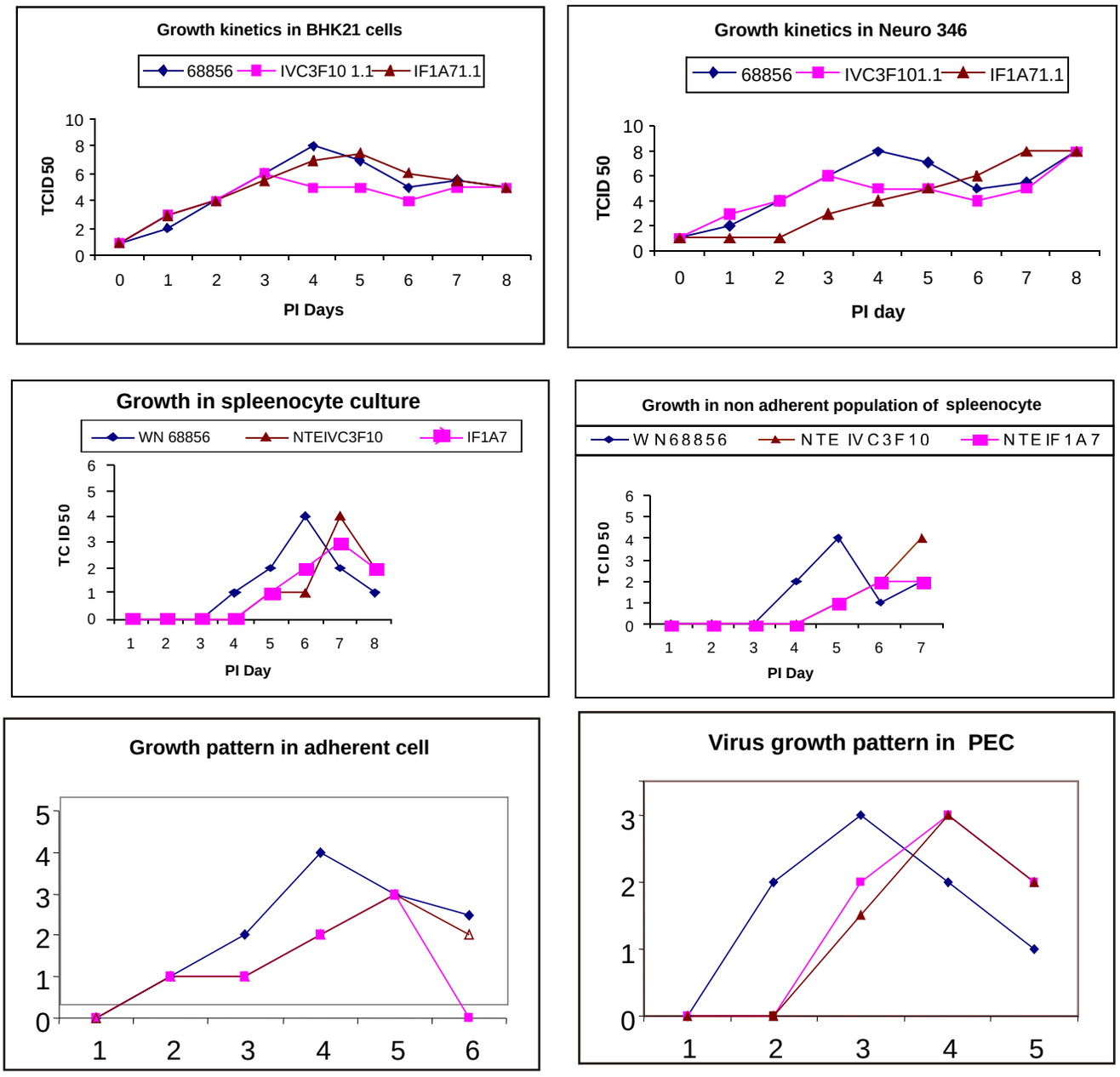


Fig. 8. Altered cell tropism of mutant viruses.

It was observed that the peripherally pathogenic WN 68856 shows significant titers within 24 hrs and 48 hrs and attains its maximum peak by 72 hrs in both splenocytes and PEC while the two mutant strains exhibit a very low titer in 24, 48 & 72 hrs and attain their maximum peaks only by 96hrs **(Fig.8)**. Thus the peripherally less pathogenic WNV exhibited a delayed release from the infected cells.

Although both the mutants retained high level of infectivity in Baby Hamster Kidney (BHK 21) cells, IF1A71.1 mutant exhibit delayed release in mouse neuroblastoma (Neuro 346) cells as compared to wild type and IVC3F101.2 mutant viruses. It appears that efficiency of replication in Neuron346 is likely to be a primary determinant of virulence.

When we compared growth pattern in splenocyte culture and its adherent, non-adherent populations, parent and mutant viruses exhibited different kinetics of replication **(Fig. 8)**. It appears that *In vitro* a wide variety of cell types successfully supports the virus replication, possibly through use of a variety of receptors of variable efficiency. It is possible that by releasing the higher amount of virus at the primary multiplication site in early phase of the infection

might be responsible for entry of the virus in brain. In addition, higher growth rate of wild type virus in neuronal cells would also contribute to the peripheral pathogenicity of the virus. Thus limiting the growth at the primary multiplication site (PEC) might be responsible for loss of pathogenicity.

Findings

Changes in amino acid domains of the E- protein might change the native conformation of the protein resulting in altered binding affinity of E- protein with its receptor. The delay in replication for mutant under conditions of high as well as low MOI suggests that the efficiency of penetration may differ as well.

Evaluation of the protective ability of immune cells against the challenge of WN virus strains Eg - 101 and 68856

SM Biswas, GN Sapkal, P. Lalwani, VM Ayachit, VP Bondre and MM Gore.

Background

Differences in genetic and antigenic nature of Egyptian prototype strain Eg-101 and Indian pathogenic strain 68856 prompted us to evaluate the role of immune splenocytes in protection against WNV by adoptive transfer.

Objective

- Determining role of the immune splenocytes in protection against WNV infection in mice.

Work done

Spleens from mice (BALB/c) immunized with three doses of Eg-101 (1000 LD_{50}) strain were harvested on 7th day post final immunization. Approximately 5x10^7 immune cells were adoptively transferred to irradiated mice via i.v. route which were challenged after 24 hrs with 50 LD_{50} of either Eg-101 or 68856 strains. Mice were observed up to 28 days for survival of challenge.

Findings

Challenge by homologous strain (Eg-101) induced 60% protection while in heterologous challenge by 68856 strain showed only 37.5% protection. Similarly challenge by JEV strain 733913 by i.p + ic routes showed only 25% protection.

Transfer of CD4 depleted and CD8 depleted cell population of WNV E-101 immune mice

Lymphocytes subpopulations isolated from immune splenocytes were separated by magnetic cell separation method and used for cell transfer and challenge studies**.** 75% of mice, which had been transferred with CD4+ depleted splenocytes, were protected from homologous WN E-101 challenge. CD8+ depleted splenocytes were, however not effective in mediating protection against homologous challenge WN E-101 **(Fig. 9)**

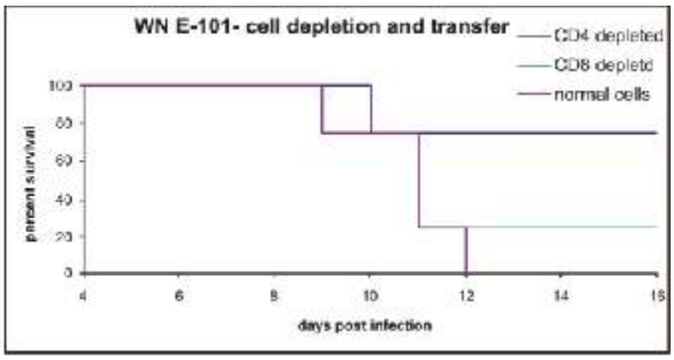


Fig. 9. Survival curve of mice lethally challenged with WNV Eg101 after adoptive transfer of CD4 and CD8 depleted splenocytes.

Immunoprotective mechanism in mouse model of JE virus

M Biswas and MM Gore.

Determining the role of CMI in protection against JEV infection in mice.

Mechanism of protection from lethal challenge of JE virus is less understood. Using cell transfer methods role of CMI can be studied.The study would be useful in understanding JE pathogenesis and immunodominant epitopes.

Objectives

To understand the role of CMI in protection of JE in mice.

Work done

Determination of virus titers in organs of mice receiving immune and non-immune splenocytes:

Viral replication in the peripheral organs and in the CNS of mice receiving immune or non-immune splenocytes was studied. Viral loads were detected by real time PCR using JEV specific primers and probe.

Fig. 10a

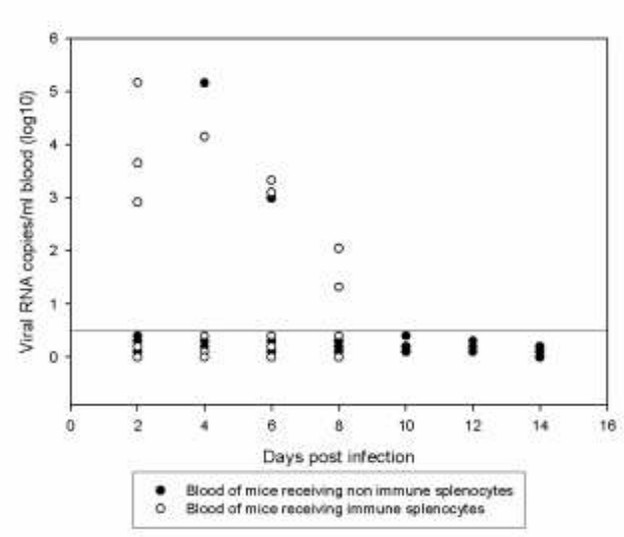


Fig. 10b

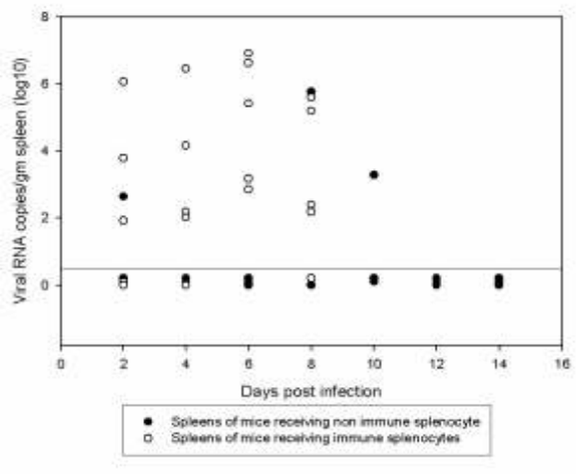


Fig. 10c

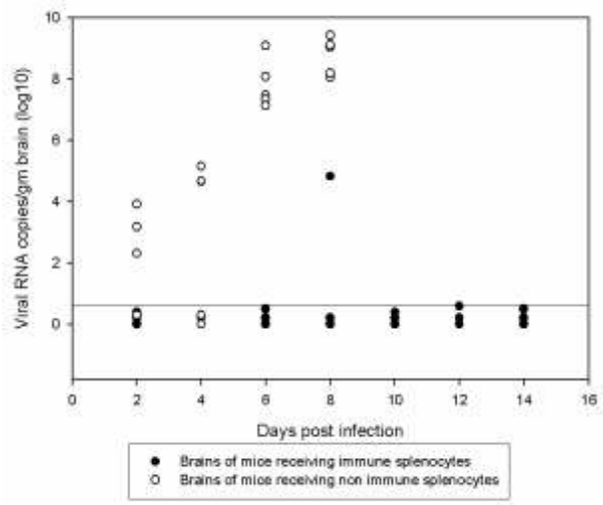


Fig.10. Scatter plots showing viral RNA copies in the a) Blood, b) Spleen, and c) Brain of mice receiving either JEV non-immune or immune splenocytes, taken at various time points PI. Each circle represents pooled organs from 2 mice.

Increased production of pro-inflammatory cytokines in infected mice:

Th1 and Th2 cytokines produced in response to JEV infection were determined by the CBA kit in the sera of mice receiving either immune or non-immune splenocytes. Higher levels of pro-inflammatory cytokines TNF-α ($p< 0.05$) and IFN-γ were observed in the sera of infected mice as disease progressed. TNF-α and IFN- γ expression levels seemed to parallel the progression of disease in non-immune mice, with baseline levels on day 2 PI continuously increasing, concurrent with the death of the animal. Levels of IL-2, IL-4 and IL-5 were much lower and peaked earlier during the course of infection (4th day PI), reaching basal levels by days 6 and 8 PI. Late in the course of infection, expression of IL-4 and IL-5 was seen to decrease in the sera of mice receiving non-immune splenocytes

Fig. 11a.

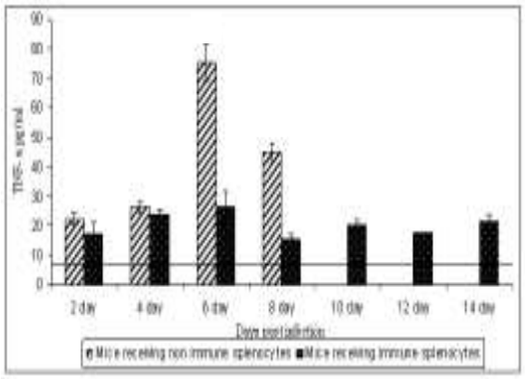


Fig. 11b.

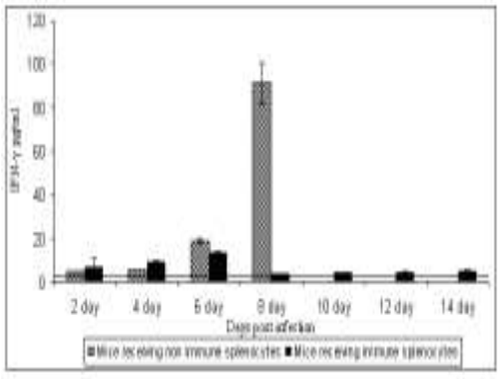


Fig. 11c.

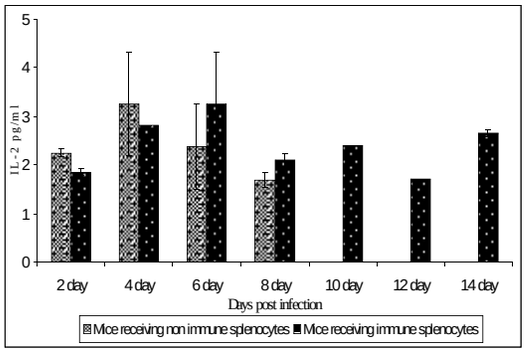


Fig. 11d.

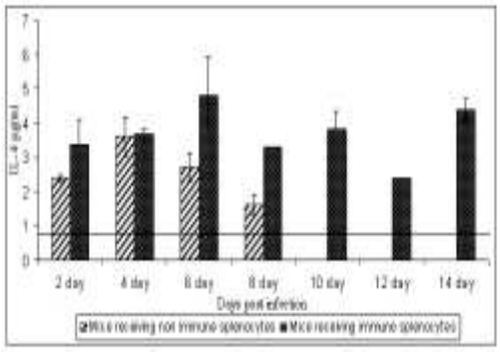


Fig. 11e.

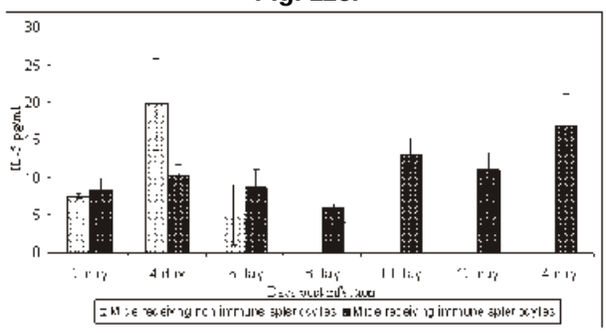


Fig. 11. Induction of pro and anti-inflammatory cytokines a) TNF- ? b) IFN- ?c) IL-2, d) IL-4, and e) IL-5 in the sera of mice receiving either non-immune or immune splenocytes.

Increased production of IgG1 antibodies in mice receiving immune splenocytes.

Induction of the antibody response after JEV infection in mice receiving either non-immune or immune cells was determined. In non-immune mice, IgM antibody reached titres of 1/400 by day 8 PI **(Fig. 12).** In mice receiving immune splenocytes before challenge with JEV **(Fig. 13)**, an elevated level of IgM antibody was observed with titres that were higher overall (1/560) than those observed in mice receiving non-immune splenocytes.

Antibody isotyping of IgG subtypes in mice receiving either non-immune or immune splenocytes revealed a significant increase in levels of IgG1 in the group that received immune splenocytes **(Fig. 13).**

IgG1/IgG2a ratios in mice receiving either non-immune or immune cells were calculated **(Fig. 14).** In contrast to non-immune mice, which showed a constant IgG1/IgG2a ratio at all time points PI, mice receiving immune splenocytes showed an increased ratio indicating increased IgG1 titres that suggested a switch towards a Th2 response.

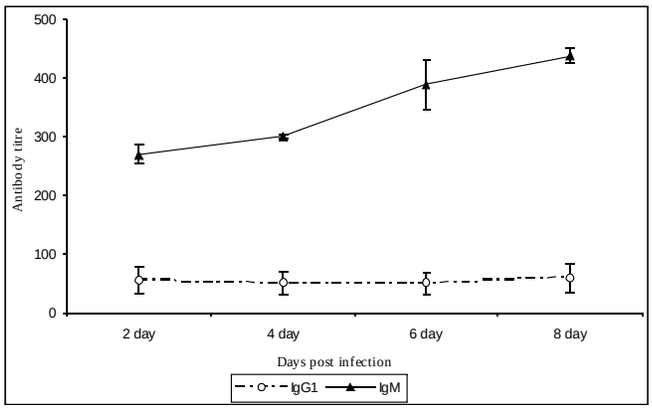


Fig. 12. IgM and IgG1 titres from pooled sera of JEV infected mice, collected at different time points post infection.

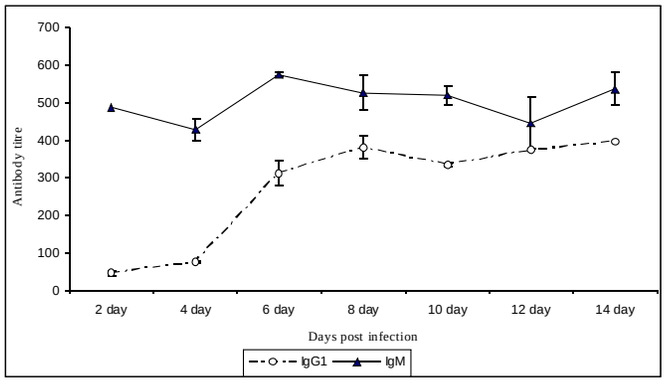


Fig. 13. IgM and IgG1 titres from pooled sera of mice subjected to adoptive transfer with JEV immune splenocytes, collected at different time points post infection.

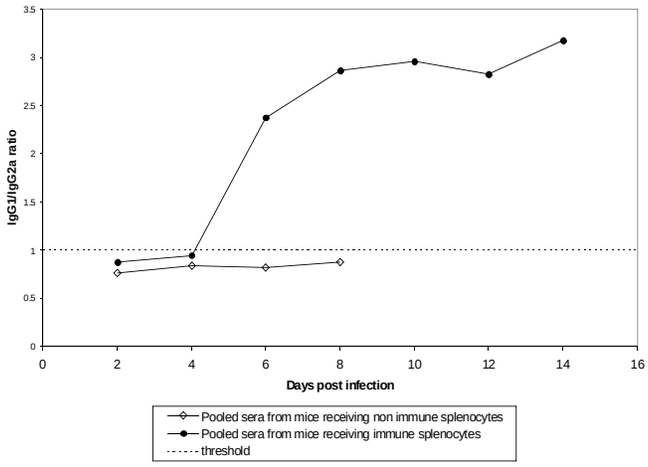


Fig. 14. IgG1/IgG2a ratios from pooled sera of mice receiving either JEV non-immune or immune splenocytes.

Differential expression of genes in mouse brain infected with JE virus.

SM Biswas, Rashmi Singh, V Vipat, MM Gore, D Ghosh

Our aim was to identify key molecules involved in the early response to JE infection in the brains of 14 day old mice. An extraneural route of infection was employed as it mimics the conditions of natural infection in man. Using the Discovery Mouse Chip, specific for 381 genes/probes, we identified 43 upregulated and 65 downregulated genes, whose expressions were modulated early during JE infection. Validation of the microarray data generated was done using Real time Sybr green RT PCR and RT PCR on a small subset of these genes.

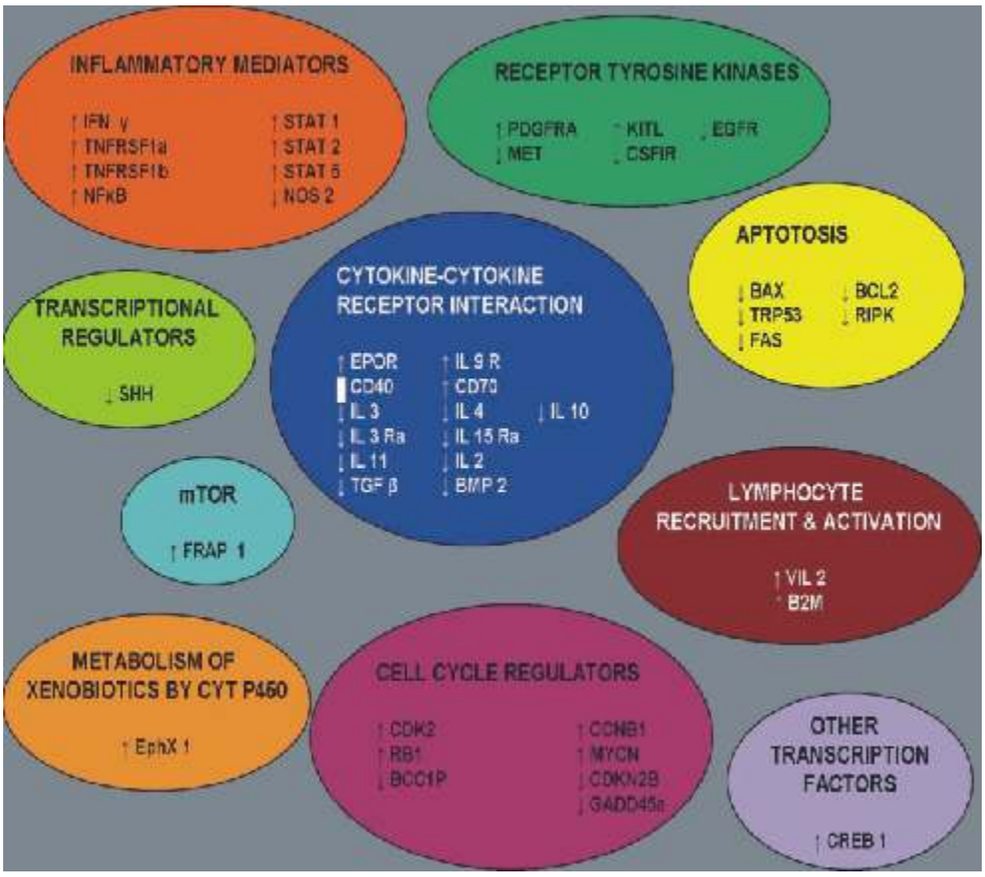


Fig.15. Grouping of the genes up and down-regulated in JEV infected mouse brain, upon microarray analysis, into functional groups.

Table.1. In the microarray analysis, 43 genes were upregulated out of 381 probes, of which 23 genes of our interest are represented in the table along with corresponding fold changes.

UPREGULATED GENES

GenBank Accession No.	Gene Name	Fold Change	GenBank Accession No.	Gene Name	Fold Change
NM_009283	STAT 1	20.436	NM_020009	FRAP1	38.791
AF187231	STAT 2	9.650	NM_008374	IL9R	14.09
NM_011488	STAT 5a	2.447	NM_009029	RB1	10.59
K00083	IFN-γ	18.354	NM_010301	GNA11	2.165
NM_011609	TNFR1	2.291	NM_011611	CD40	8.515
NM_011610	TNFR2	1.339	NM_011617	CD70	3.311
NM_009735	B2M	10.189	NM_009510	VIL-2	3.167
NM_008689	NFkB1	4.31	NM_016756	CDK 2	1.164
M95106	CREB 1	17.787	X64713	CCNB 1 (Cyclin B1)	1.407
NM_010149	EPOR	2.396	NM_008709	MYCN	4.636
NM_011058	PDGFRA	2.493	NM_021274	CXCL10	1.356
NM_013598	KITL	1.513			

Table.2. In the microarray analysis, 65 genes were downregulated out of 381 probes, of which 27 genes of our interest are represented in the table along with corresponding fold changes.

DOWNREGULATED GENES

GenBank Accession No.	Gene Name	Fold Change	GenBank Accession No.	Gene Name	Fold Change
NM_007527	BAX	0.113	AK010684	BCCIP (p21binding)	0.409
NM_007987	FAS	0.189	NM_011640	TRP53	0.683
NM_009741	BCL2	0.315	U41504	IL2	0.318
NM_009068	RIPK1	0.114	NM_010556	IL3	0.202
NM_007912	EGFR	0.1761	NM_008369	IL3Ra	0.8325
NM_007779	CSF1R	0.1639	NM_021283	IL4	0.326
NM_022994	DAP3	0.218	NM_010548	IL10	0.004
NM_009397	TNFAIP3	0.148	NM_010549	Il11RA1	0.236
NM_009424	TRAF6	0.613	NM_008350	IL11	0.540
NM_010730	ANXA1	0.599	NM_008354	IL12RB2	0.0630
NM_009367	TGFB 2	0.239	S80963	IL13RA1	0.5282
X76290	SHH	0.258	NM_008358	IL15Ra	0.542
NM_007836	GADD45a	0.213	NM_010927	NOS2	0.808
NM_007670	CDKN2b (p15)	0.6535			

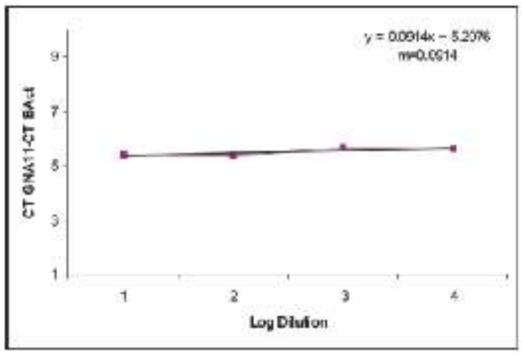


Fig.16. Calculation of relative SYBR Green PCR efficiency using serial 10 fold dilutions of the reference gene, í-actin and a target gene, GNA-1 1. A plot of the ?Ct versus log dilution gave a slope, m< 0.1, reflecting optimal PCR efficiency for both genes.

Table.3. Real Time SYBR Green data showing fold up regulation of genes from brains of mice on the 3rd day post infection as compared to sham-inoculated controls. Standard deviation for each gene value is indicated. Data represents an average of 4 datasets, each assay being performed with a pool of 2 mice brains. For an individual experiment, each gene was assayed in triplicates.

Up regulated Genes

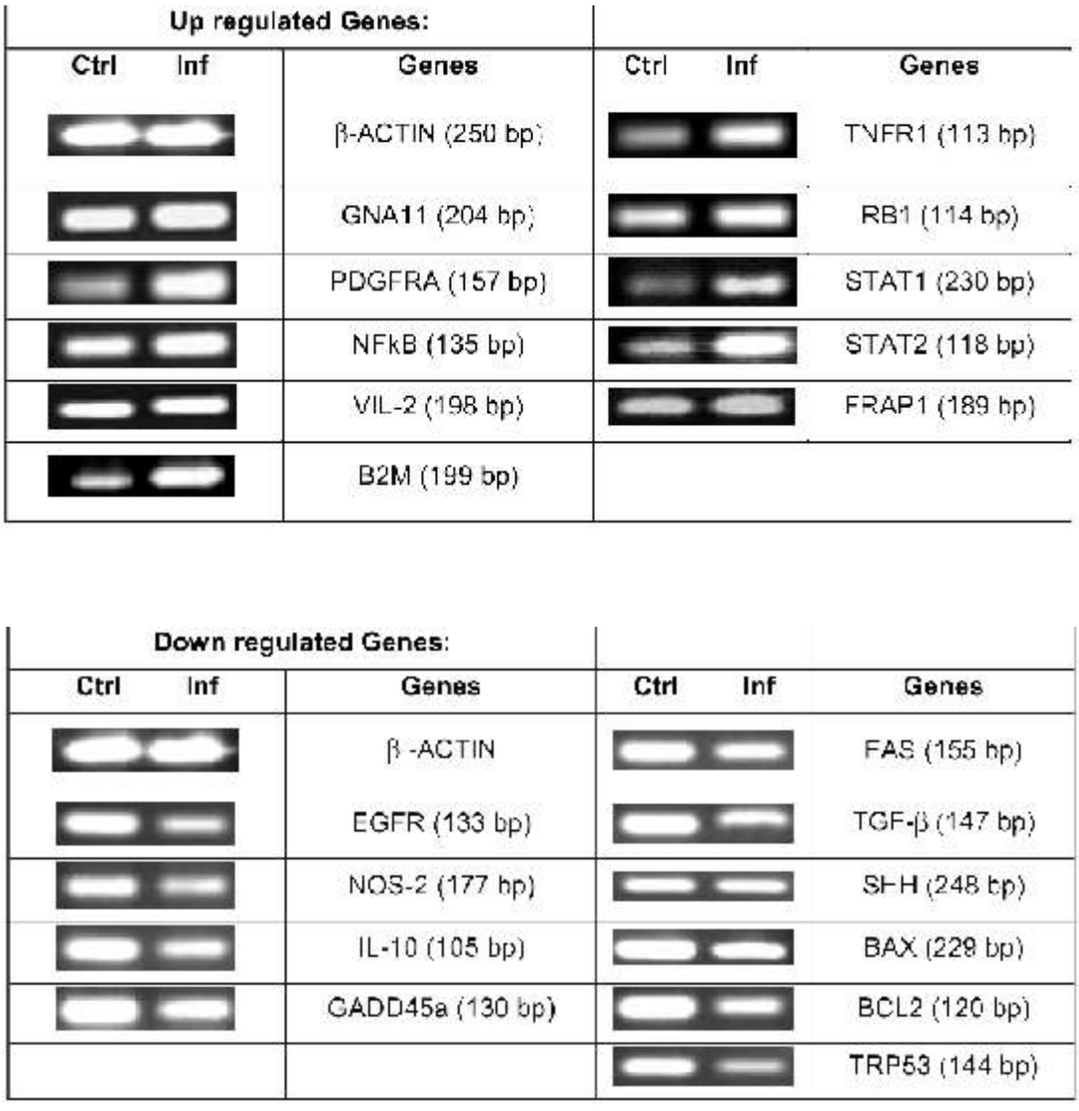
Gene Name	Fold Change (2-ΔΔCT)	StDev
GNA-11	1.227945	±0.157893
PDGFRA	1.137266	±0.144667
B2M	2.982893	±1.011186
VIL-2	1.484733	±0.487663
NF-kB	1.529815	±0.225173
TNFR1	1.209497	±0.16471
RB1	1.48855	±0.326664
STAT1	13.51539	±6.348694
STAT2	3.618133	±0.788336
FRAP1	0.957407	±0.26452

Table.4. Real Time SYBR Green data showing fold down regulation of genes from brains of mice on the 3rd day post infection as compared to sham-inoculated controls. Standard deviation for each gene value is indicated. Data represents an average of 4 datasets, each assay being performed with a pool of 2 mice brains. For an individual experiment, each gene was assayed in triplicates.

Down regulated Genes

Gene Name	Fold Change (2-ΔΔCT)	StDev
EGFR	0.5	±0.141421
NOS2	0.576874	±0.380064
IL-10	0.322266	±0.011054
GADD45a	0.925169	±0.341354
FAS	0.881044	±0.105838
TGF-β	0.691948	±0.390401
SHH	1.37	±0.04
BAX	0.700355	±0.016994
BCL2	0.873903	±0.335566
TRP53	0.816196	±0.0884

Fig.17. Up regulated and down regulated genes.



Post marketing study to evaluate the safety and immunogenicity of a single dose of JE vaccine at Burdwan, West Bengal

MM Gore, VM Ayachit, D Gangale, GN Sapkal

Background

In Burdwan West Bengal, 361 children were vaccinated with SA 14-14-2 vaccine in June 2006. Blood samples were collected on day 0, 28, 6 months and one year after vaccination. Sera were separated at the site and sent to NIV, for testing of neutralizing antibody response to Indian strain of JE (057434), and Beijing strain (Thailand) of JE virus. Serum virus neutralizing antibody titers were determined by carrying out PRNT with above named viruses. All the samples were stored at -20°C at NIV.

1. Day 0 samples

In order to assess the background immunity to JE and WN viruses in the children from Burdwan region prevaccination sera were tested for neutralizing antibodies against JE (Indian and Beijing) strains and WN viruses. Following results have been obtained.

Prevaccination N'Abs against JE strain from		
	India	Beijing
Total tested	345	345
Positive >10	159	87
% positive	46.08	25.29
Mean Ab titer	17.81	11.89

Individuals having <10 N'Ab titer on day 0 were grouped together while those with day o N'Ab titer >10 were grouped separately. Virus neutralizing antibody titers were determined after 28 days, 6 months and 1 year following the single dose of JE vaccine. Results obtained using Indian JE (057434) and Beijing JE virus are as follows:

Table. 1. Virus neutralizing antibody titers in 363 vaccinees tested against Indian JE virus strain 057434.

Days Post Vaccination	0 day	28 day	6 months	1 year
Group I: Individuals negative for anti JEV antibodies on day 0 (N= 193)				
Responders (%)		123/190 (64.74)	81/190 (42.63)	63/185 (33.68)
Geo Mean Titer	8.06 ± 1.31	21.10 ± 81.74	11.77 ± 129.11	9.01 ± 26.97
Group II: Individuals positive for anti JEV antibodies on day 0 (N= 167)				
Responders (%)		136/159 (85.53)	117/162 (72.22)	88/161 (54.66)
Geo Mean liter	24.59 ± 99.46	102.25 ± 2721.80	36.18 ± 286.03	22.26 ± 127.60
Total of Group I & II				
Responders (%)		259/349 (74.21)	198/352 (56.25)	152/351 (43.30)

Table. 2. Virus neutralizing antibody titers in 363 vaccinees tested against JE virus strain Beijing.

Days Post Vaccination	0 day	28 day	6 months	1 year
Group I: Individuals negative for anti JEV antibodies on day 0 (N= 262)				
Responders (%)		77/252 (30.56)	40/255 (15.69)	21/256 (8.20)
Geo Mean Titer	7.16 + 1.19	9.28 + 43.53	7.23+ 34.64	6.33 - 17.94
Group II: Individuals positive for anti JEV antibodies on day 0 (N= 100)				
Responders (%)		72/96 (75.00)	56/97 (57.73)	53/96 (55.21)
Geo Mean titer	27.04 = 51.11	58.04 ± 372.41	25.13 ± 86.39	25.99 ± 116.59
Total of Group I & II				
Responders (%)		149/348 (42.82)	96/352 (27.27)	74/352 (21.02)

A prospective open-label, uncontrolled, single-centre, evaluation of viraemia in healthy flavivirus seronegative adults after primary vaccination with JE SA14-14-2 live attenuated vaccine (JEATTE/IND/01- PUNE)

MM Gore, VS Padbidri, VP Bondre, GN Sapkal, PV Fulmali, V. Shankaraman, G Jogdand, Y Rajmane, VM Ayachit, D. Gangale

Objectives

Primary objective

- To determine levels of viraemia after administration of a single dose of live attenuated SA14-14-2 Japanese encephalitis vaccine in adult subjects between days 1-8 and day 15.

Secondary objectives:

- To determine neutralizing antibody response at 30 days, 6 months and one year after administration of a single dose of live attenuated SA14-14-2 Japanese encephalitis vaccine in adult subjects.
- To evaluate safety from the time of administration of a single dose of live attenuated SA14-14-2 Japanese encephalitis vaccine in adult subjects till one year.

1. Enrolment procedure:

The steps enumerated in the Protocol were followed for recruitment. The enrolment commenced at the site on 28 May 2007 and ended on 01 Feb 2008. A total of 287 people between the age of 18 and 40 years and both genders, were screened.

The subjects were first screened to see if they fulfilled the Inclusion and Exclusion criteria. They were then explained the project and their participation. They were also provided with the Patient Information Sheet and Informed Consent Form. After the subject signed the Informed Consent Form the clinical examination was carried out and his/her blood sample was obtained to determine the antibody status for Japanese encephalitis (JE), West Nile (WN) and Dengue (DEN) antibodies by performing the HI test. Values above 1:10 were considered as positive. The sera of those subjects who had HI titres of $\leq$ 1:10 were further tested in PRNT. Here also, the cut off point was a value above 1:10. Subjects who had values of $\leq$1:10 in both the tests were eligible to receive the vaccine.

The eligible vaccinees were then bled again and bio chemical/haematological tests performed, along with ELISA tests for HIV-1, Hepatitis B virus surface antigen and Hepatitis C virus. Urinalysis was also carried out in female vaccinees for routine and pregnancy tests. All these procedures were carried out during Visits 1 and 2.

On the day of vaccination (Visit 3) the subjects were given the Informed Consent Form for perusal and signature. Physical examination and urinalysis was carried out. They were assigned to one of the 3 groups of vaccinees. After administration of vaccine, they were kept under observation for half an hour. They were then provided with a clinical thermometer, measure tape and Daily Diary Card and explained how to fill it up.

2. Vaccinee groups:

There were three groups for the study.

Group 0 consisted of 12 vaccinees. They were required to report everyday, from Day 1 to Day 7, Post vaccination. (Visit 4 - Visit 7)

Group 1 consisted of 12 vaccinees. They were required to come on alternate days on odd days - Day 1, Day 3, Day 5 and Day 8 post vaccination (Visit 4 - Visit 7)

Group 2 consisted of 11 vaccinees. They were required to come on alternate days on even days - Day 2, Day 4, Day 6 and Day 8 post vaccination (Visit 4 - Visit 7)

The first batch of vaccinees was administered the dose on 26 June 2007. While the last batch was vaccinated on 18 Feb 2008.

3. Severe adverse events:

There was only one severe adverse event (SAE) reported so far, in the study. DTB, a male 20 years of age, Enrolment No. 019 from Group 1, received the vaccine on 30 Aug 2007. On 24 Nov 2007 he had moderate fever with chills (101^0) burning of eyes and giddiness, for which he was hospitalised on 27 Nov 2007 He was given symptomatic treatment and anti malarial therapy. The diagnosis was "viral fever with conjunctivitis". He recovered rapidly and was discharged on 30 Nov 2007. Recovery was complete and without any sequelae.

As per the Investigator the SAE was NOT RELATED to the vaccine.

The SAE was reported to the Local Safety Monitor by telephone on the afternoon of 30 Nov 2007. This was followed by sending her all the relevant information and proforma by fax on 01 Dec 2007.

Adverse events (AEs):

Several AEs were reported, which are summarized as below in the table.

Table. Summary of adverse events in vaccinees.

Sr. No.	Enrol No.	Vaccinee Group	Nature of AE
1	004	Gr. O	Headache
2	006	Gr. O	Headache, Weakness
3	008	Gr. O	Rhinitis, Pharyngitis Came with C/o generalised itching with dryness and tenderness in rt. hypochondriac region. Clinically there were no signs and USG abdomen was not significant. His SGOT (Normal upto 30 Units/L) and SGPT (Normal upto 40 Units /L) were found to be raised during visit 8 - SGOT 53 units /L and SGPT 101 Units/L. Clinically, these were not found to be significant
4	010	Gr. O	Headache
5	014	Gr. 1	This vaccinee had no complaints but his SGOT and SGPT levels were found to be raised Visit SGOT SGPT V2 49 73 V8 114 183 These were not clinically significant
6	015	Gr. 1	This vaccinee complained of weakness and loss of power in the left hand on 1/10/07. He was referred to the Neurologist. He was diagnosed to have Lt. radial neuropathy and advised physiotherapy and vitamins.
7	029	Gr. 2	Headache

All these AEs were not related to the JE vaccine administration.

4. Viremia in vaccinees

Level of Viremia was tested in vaccinees on different days as mentioned above. The sera were separated and titrated on BHK cells, which are capable of supporting SA 14-14-2 virus growth. In addition virus copy number was determined by Real Time PCR assay on the whole blood.

It was observed that only one volunteer Enrolment No. 002 showed detectable copy numbers on the 8th day of vaccination. (242 copies /mL). All other subjects did not show any detectable virus level in either serum or whole blood.

5. Antibody response to vaccination.

Blood samples were collected on 0, and 30 day after vaccination. Analysis of antibody titers obtained 30 days after vaccination revealed that out of 35 subjects 9 did not sero convert (Sero-conversion in 74.28%). Titers are in the range of 10.43- 133.03. Assay for antibody titers in serum samples collected after six months after vaccination has been completed in 20 subjects. Only 1 out of 20 subjects has shown antibody titers >10.0

Further work

Samples would be collected for testing the antibody titers after six months and one year for the remaining vaccinees. The clinical assessment of the subjects at the end of this period would be analysed. The data would be analysed by the Data Management Team of the study. The report would be then submitted.

Selective Expression of Recombinant Viral Protein in Immunocompetent Cells

MF Ahsan, MM Gore

Background

In conventional DNA vaccines the expression are controlled by a nonspecific (CMV) promoter making it possible to express the protein in all the cells, which get transfected including that of nonprofessional APCs. This might affect the overall intensity and longevity of immune response *in-vivo*. Hence, the approach is to express the antigen only in professional APCs, this could be achieved by using cell specific promoters.

Objective

Cloning and Expression of structural genes of Japanese Encephalitis Virus under the control of immune cells specific promoter. Study immune response and protection *in-vivo* when those recombinants are used as a plasmid vaccine.

Work done

Employing Reverse Transcriptase PCR, E-gene with and without transmembrane sequence of Japanese encephalitis virus (strain - 733913) was amplified. Amplicons were cloned into pGEM T Easy vector and transformed to JM 109 competent cells. Gel of isolated plasmid of ligated product of pGEMT (3KB) and PCR product of complete and truncated E

JEV E-gene (with and without transmembrane sequences) from TA Cloned pGEM T Easy vector was excised and cloned in pAcGFP1-N1 expression vector at EcoR I site and transformed into JM 109 competent cell. Recombinant vector was isolated by plasmid mini prep method and JEV E gene was confirmed by restriction digestion analysis. By sequencing it was observed that the start and stop codon is in-frame. Both the codons were

included in primers at the time of primer design. Digestion pattern of both the clones (Complete and Truncated E) when digested with two different Restriction enzymes as a confirmation of insert. The clones were further confirmed through sequencing.

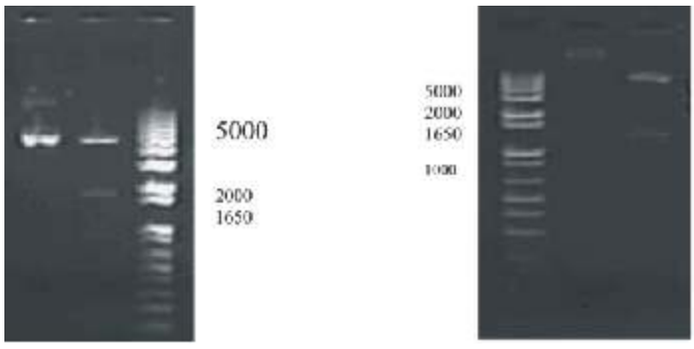


Fig.18. Cloning of E gene pAcGFP1-N1 vector.

1	Undigested vector of 4.7 KB	1	1 Kb+ DNA Ladder
2	Clones (complete E protein) digested with SacI fragments size of 4999 & 1812 bp.	2	Undigested cloned vector of 7 KB
3	1 Kb+ DNA Ladder	3	Clones (Truncated E protein) digested with Hpa I fragments size of 1263 & 5242 nt.

Protocol was standardized with Lipofectamine 2000 for the Transfection of plasmid (pAcGFP1-N1) with CMV as a promoter in Vero and RAW 264.7 cell lines, Opti-MEM was used as a medium. After 24 hours of post transfection, cells were observed for fluorescence under fluorescent microscope. As a negative control, cells without plasmid were transfected with Lipofectamine 2000. For macrophage specific promoters, macrophage specific markers have been selected and the sequence retrieved from GenBank. Primer would be designed using Primer Express version 2.0 and various online available tools (Primer3, Oligo-analyzer, etc.)

A B

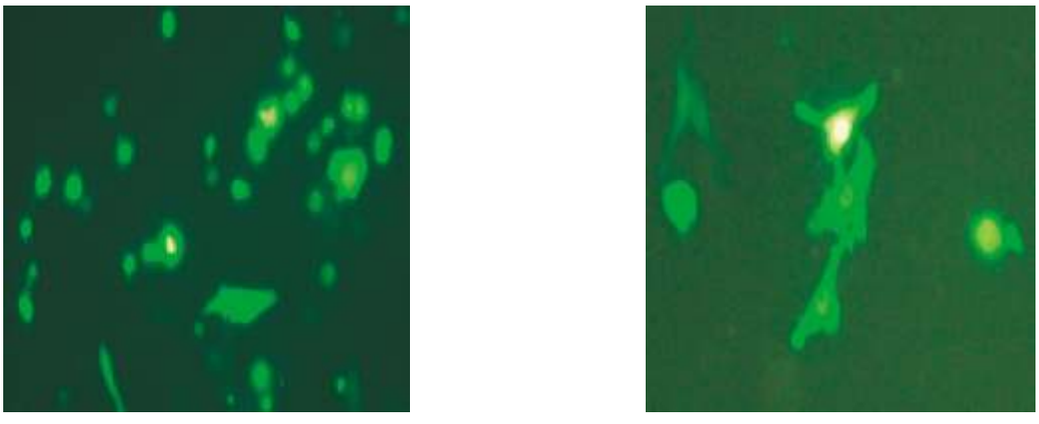


Fig.19. Expression of GFP post transfection in (A) Vero cells and (B) RAW 264.7 cell line

Findings

Cloning of truncated and complete envelope protein of JEV has been achieved. Standardization of transfection using Lipofectamine 2000 in Vero and RAW 264.7 cells has been achieved.

Development of molecular techniques for the rapid detection of agents infecting the central nervous system

VP Bondre, PV Fulmali, GN Sapkal, V Shakaraman, and MM Gore.

Background

Acute viral encephalitis is known to be caused by a wide range of viruses either in sporadic or in outbreak forms. Globally identified viral etiological agents include Herpes, Entero, Alpha, Influenza A, Rabies, Human Immunodeficiency and Flaviviruses. In India, Japanese encephalitis (JE) and West Nile viruses co-exist in many areas. Some of the cases go undiagnosed because of the unavailability of the sensitive diagnostic techniques. Hence, there is need to developed highly sensitive diagnostic methods. As a first step flavivirus specific semi nested RT PCR has been developed.

Objective

- Development of a highly sensitive semi nested RT-PCR based diagnosis for Flavivirus detection.

Work done

Flavivirus diagnosis

A one step reverse transcription polymerase chain reaction (RT-PCR) followed by seminested PCR was developed for Flavivirus detection and standardized using laboratory strain of titrated Japanese Encephalitis, West Nile Virus and Dengue 2 and Bagaza virus. The other viruses viz, Chikungunya (Alphavirus) and Chandipura (Rhabdovirus), negative human clinical specimens and normal vector mosquitoes were also included to check for non specific amplification. Sensitivity of RT-PCR and semi-nested PCR was determined by comparing the endpoint dilution of plaque titrated different flaviviruses stocks grown in cell culture and infected mouse brain suspensions. The sensitivity RT-PCR using serially diluted WN (68856) virus (7.3 $\log_{10} LD_{50}$/ml) was determined.

Findings

The one step RT-PCR specifically detects WNV and does not cross-react with other viruses (Alphaviruses and Rhabdoviruses), however, its sensitivity to detect titrated WNV virus falls between 100-1000 plaque-forming units (pfu). The semi nested PCR of products amplified in one step RT-PCR specifically detected 1-10 pfu of WNV virus. Additionally, the sequence confirmation of WNV (68856) of semi nested PCR amplified product has confirmed the specificity of our semi nested PCR. The assay is being used for diagnosis of specimens from encephalitis cases.

Publications

- Bondre VP, Jadi RS, Mishra AC, Yergolkar PN, Arankalle VA. West Nile virus isolates from India: evidence for a distinct genetic lineage. **J Gen Virol**, 2007; 2007 Mar; 88(Pt 3): 875-84.
- Dewasthaly SS, Bhonde GS, Shankarraman V, Biswas SM, Ayachit VM, Gore MM. Chimeric T helper-B cell peptides induce protective response against Japanese encephalitis virus in mice. **Protein Pept Lett,** 2007; 14 (6): 543-51.
- Sapkal GN, Wairagkar NS, Ayachit VM, Bondre VP, Gore MM. Detection and isolation of Japanese encephalitis virus from blood clots collected during the acute phase of infection. **Am J Trop Med Hyg,** 2007 Dec; 77(6): 1139-45

Workshops / Conferences / Seminar / Meetings attended

MM Gore

- Oral presentation on "Differential dominance of $CD8^+$ and $CD4^+$ cell population in protection from Japanese encephalitis and West Nile virus infection in mice" in International conference organized by Indian Virological Society, New Delhi at PUSA campus during December 11 -13, 2007.

VP Bondre

- Oral presentation on "Molecular epidemiology of Japanese encephalitis virus in India: Scenario during last fifty years (1956 2006)" in International conference organized by Indian Virological Society, New Delhi at PUSA campus during December 11 -13, 2007.
- Invited lectures "Epidemiology of Japanese encephalitis: Global and National perspectives" in State level Workshop on Japanese encephalitis, organized by National Vector Borne Disease Control Programme, Govt. of India, the Department of Family Welfare and Arogyakeralam at Tiruvananthapuram (Kerala) on March, 12 -14, 2008.
- Invited lectures "Viral agents causing encephalitis" in State level Workshop on Japanese encephalitis, organized by National Vector Borne Disease Control Programme, Govt. of India, the Department of Family Welfare and Arogyakeralam at Tiruvananthapuram (Kerala) on March 12 -14, 2008.

GN Sapkal

- Training course in Flowcytrometry at New Delhi during July 2-5, 2007.

Patent

Indian, Vietnam, Korea, US Patent granted to Gore, M. M., Dewasthaly, S. S., Kolaskar, A. S., Kulkarni-Kale, U. D. (April, 2007). Chimeric T helper B-cell peptide as a vaccine for flaviviruses.