SURVIVAL OF MICE IMMUNIZED WITH MONOCLONAL ANTIBODIES AGAINST GLYCOPROTEIN E OF JAPANESE ENCEPHALITIS VIRUS BEFORE OR AFTER INFECTION WITH JAPANESE ENCEPHALITIS, WEST NILE, AND DENGUE VIRUSES

A.K. GUPTA, V.J. LAD, A.A. KOSHY

National Institute of Virology, Indian Council of Medical Research, 20-A, Dr. Ambedkar Road, P.B. No. 11, Pune, 411001, India

Received June 8, 2007; accepted October 1, 2008

Summary. – In the present study, the effect of monoclonal antibodies (MAbs) against glycoprotein E (gE) of Japanese encephalitis virus (JEV) strain 733913 administered 1 day before or 2 days after intracerebral (i.c.) challenge with West Nile virus (WNV) strain 68856 or Dengue virus (DENV-2) strain P23085, was studied in mice. Furthermore, two JEV strains belonging to group II (strains 641686 and 691004) that have lost reactivity against virus-specific MAbs were also used in passive immunization experiments. MAbs as ascitic fluids were administered intraperitoneally (i.p.) in mice. Hemagglutination-inhibition- (HAI) positive JEV-specific (Hs-3) MAbs given 2 days after the virus infection showed reduced mortality along with increased survival of mice challenged with WNV or with DENV-2. Also the HAI-positive flavivirus cross-reactive (Hx) MAbs produced a marginal increase in the survival of mice challenged with both JEV strains 641686 and 691004 belonging to the group II. As the MAbs reacting with HAI-positive JEV-specific (Hs) and HAI-negative JEV-specific (NHs) epitopes were neutralizing and protective in mice against JEV strain 733913 challenge, the results indicated presence of the cross-protection phenomenon that might be occurring in some of the localities endemic for the three closely related flaviviruses.

Key words: flaviviruses; monoclonal antibodies; cross-protection; Japanese encephalitis virus; West Nile virus; Dengue virus

Introduction

Japanese encephalitis caused by a mosquito-borne JEV (the family *Flaviviridae*, the genus *Flavivirus*) has gained considerable public health importance both in Southeast and Western Pacific regions (Monath and Heinz, 1996; Vaughn and Hoke, 1992; Rodrigues, 1984). Since 1995, the disease has also emerged in non-Asian region such as Northern Australia (Hanna *et al*., 1999, 1996). The situation in Southeast Asia, however, is further complicated by overlapping epidemics of JEV and DENV as well as by sporadic cases of WNV detected in some of the affected areas (George *et al*., 1984). The simultaneous incidence of these three closely related flaviviruses in some parts of Southeast Asia particularly in India has been of epidemiological interest due to the cross-

E-mail: drashok.gpt@gmail.com; fax: +91-2422-273413.
Present address: Rural Medical College, Pravara Institute of Medical Sciences, Loni – 413736, India.

Abbreviations: ADVE = antibody-dependent virus enhancement; AF = ascitic fluid; AST = average survival time; CHIKV = Chikungunya virus; DENV = Dengue virus; gE = glycoprotein E; HA = hemagglutination; HAI = hemagglutination-inhibition; Hs = HAI-positive JEV-specific; Hx = HAI-positive flavivirus cross-reactive; i.c. = intracerebral(ly); i.p. = intraperitoneal(ly); JEV = Japanese encephalitis virus; MAb(s) = monoclonal antibody(ies); MR = mortality ratio; NHA = HAI-negative auto-reactive; NHs = HAI-negative JEV-specific; NHx = HAI-negative flavivirus cross-reactive; N = neutralization; WNV = West Nile virus; YFV = Yellow fever virus

reactive immune response occurring in people residing in the affected localities (Carey and Myers, 1968).

The major envelope gE of flaviviruses contains most of the antigenic epitopes that induce various biological function such as hemagglutination (HA), neutralization (N) (Roehring, 1986; Monath, 1985) and shows antigenic reactivity that ranges from specific to cross-reactive (Gould *et al*., 1986; Halstead *et al*., 1984; Mathews and Roehring, 1984; Kimura- Kuroda and Yasui, 1983; Heinz *et al*., 1983; Peiris *et al*., 1982). Mapping of antigenic epitopes of gE, JEV strain 733913 (group I strain of Indian origin) employing MAbs showed existence of five domains represented by the MAb groups involving Hs, NHs, Hx, HAI-negative flavivirus cross-reactive (NHx), and HAI-negative auto-reactive (NHA) MAbs (Cecilia *et al*., 1988; Kedarnath *et al*., 1986). The MAbs reacting with Hs and NHs epitopes are neutralizing, while MAbs corresponding to Hx domain showed *in vitro* antibody-dependent virus enhancement (ADVE), in addition to the virus neutralization (Cecilia and Ghosh, 1988).

The aim of the present study was to characterize the effect of MAbs prepared against gE of JEV strain 733913 on DENV-2 (P23085), WNV (strain 68856) or group II JEV (strains 641686 and 691004) infections in mice. A group of mice infected with homologous JEV strain 733913 against which anti-gE MAbs were raised served as the positive control.

Materials and Methods

Mice. Albino mice (Webster or Swiss strain) were obtained from the Rockefeller Foundation Virus Laboratories, New York. The mice have been maintained by random breeding. Swiss 3 to 4-week-old mice were employed in the study as approved by the Institutional Animal Ethics Committee for the animal maintenance and experimental work at National Institute of Virology, Pune.

Virus strains. Two JEV strains 641686 of Indian origin and 691004 of Sri Lankan origin were characterized as group II strains on the basis of the loss of Hs epitope functional activity (Gupta *et al*., 2006a, 2000; Ghosh *et al*., 1989). Strain 68856 of WNV and strain P23085 of DENV-2 of Indian origin were employed in the protective experiments. JEV strain 733913 of Indian origin that belonged to the group I strains of JEV was included in all the experiments as a positive control. All viruses have been maintained by i.c. passage of the virus in 2-day-old suckling mice and the stock viruses were stored at -70°C.

Monoclonal antibodies. A panel of MAbs prepared against gE of JEV strain 733913 in our laboratory were classified as Hs (4 MAbs), NHs (2 MAbs), Hx (5 MAbs), NHx (3 MAbs) and NHA (2 MAbs) according to their reactivity in different assays (Cecilia *et al*., 1988; Kedarnath *et al*., 1986). All the Hs and NHs MAbs were neutralizing and have protective abilities in mice against JEV strain 733913 challenge in high (100 LD_{50}) or low dose (5 LD_{50}), respectively. Though the Hx and NHx MAbs were neutralizing, only some of them were protective in mice against the virus challenge mostly in low dose; however the former invariably depicted ADVE. All the MAbs were assayed for the antibody contents and only MAbs having high antibody titer were used in the experiments.

Ascitic fluids (AF) were obtained from pristane-primed BALB/c mice after inoculation of hybrid cells. AF produced in the mice by the inoculation of SP2/0 cells and MAb prepared against Chikungunya virus (CHIKV, the family *Togaviridae,* the genus *Alphavirus*) that did not cross-react with any of the three flaviviruses served as negative controls (Lad *et al*., 1993a).

Protective experiments. Groups of 3 to 4 week-old mice were inoculated i.c. with 100 LD_{50} of the virus. At day 2 post infection, the mice were i.p. injected with 0.1 ml of AF (diluted 1:10) containing the tested or control MAb. The inoculated mice were observed for 21 days; mortality ratio (MR) and average survival time (AST) were calculated and compared with MR and AST of control mice. MR was expressed as number of mice died/ total number of the mice tested, whereas AST was the mean survival of the mice in days, which included the dead as well as those survived the virus challenge till day 21. The mice given 0.1 ml of negative AF or MAb against CHIKV and challenged with the virus served as negative controls, whereas the mice administered anti-gE MAbs and challenged with JEV strain 733913 served as positive control.

In another series of experiment, 3–4-week-old mice were i.p. administered 0.1 ml (diluted 1:10) of immune AF or control AF (SP2/0 cells or CHIKV MAb) 1 day prior to the virus challenge (100 LD_{50} of the virus injected i.c.). MR and AST were calculated as above.

Statistics. Statistical significance of the differences for experimental mice from the control values of MR was evaluated by Chi-square test and for AST by the non-parametric method of Wilcoxon's. P values ≤ 0.05 were considered as significant.

Results

Challenge with JEV strain 733913

The effect of MAbs and homologous JEV strain 733913 was already examined in mice (Gupta *et al*., 2003) and the results were similar to those obtained in the present study. These mice challenged with homologous virus served as a positive control to the mice challenged with heterologous viruses.

The group Hs antibodies protected the mice when MAbs were given 1 day before the challenge with JEV strain 733913. Of the four Hs MAbs, the protection was most effective with MAbs Hs-4 (MR 6/20 30%, AST 15 days) and Hs-1 (MR 9/19 47%, AST 12.6 days) as compared to that of controls (MR 44/45 98%, AST 6.9–7.1 days), The protection was less effective with MAbs Hs-2 (MR 12/19 63%, AST 11.44 days) and Hs-3 (MR 12/20 60%, AST 11.26 days). Generally, the protection was significantly reduced when the Hs MAbs were given 2 days after the virus challenge. MAbs Hs-1 (MR 14/20 70%, AST 9.79 days) and Hs-2 (MR 16/20 80%, AST 8.1 days) exerted a little

Table 1. Survival of mice treated with MAbs before or after challenge with JEV strain 641686

MAb (Ig subtype)	MAb given i.p. 1 day before challenge		MAb given i.p. 2 days after challenge	
	AST in days (mean ± S.D.)	Statistical significance P	AST in days (mean± S.D.)	Statistical significance P
Hs-1 (IgG1)	6.00 ± 0.72	<0.05	5.75 ± 0.79	NS
Hs-3 (IgG2a)	6.05 ± 0.73	<0.05	6.30 ± 0.42	<0.01
Hx-1 (IgG2b)	5.95 ± 0.51	<0.01	5.95 ± 0.51	<0.05
Hx-2 (IgG2b)	5.60 ± 0.75	NS	5.85 ± 0.67	NS
Hx-3 (IgG2b)	7.65 ± 0.87	<0.01	6.45 ± 0.89	<0.01
Hx-5 (IgG2b)	5.80 ± 1.01	NS	5.40 + 0.88	NS
NHs-1 (IgG1)	8.35 ± 3.53	<0.01	6.00 ± 0.40	<0.01
NHx-3 (IgG2b)	5.40 ± 0.82	NS	7.45 ± 1.32	<0.01
SP2/0 ascites	5.30 ± 0.73	NS	5.45 ± 0.59	NS
CHICKV MAb				

NS = no significance; Hs = HAI-positive JEV-specific MAbs; Hx = HAI-positive flaviviruses cross-reactive MAbs; NHs = HAI-negative JEV specific MAb; NHx = HAI-negative flaviviruses cross-reactive MAb.

protection, but MAbs Hs-3 (MR 17/18 94%, AST 7.6 days) and Hs-4 (MR 18/19 95%, AST 6.83 days) did not have any effect. The other MAb groups, i. e. Hx and NHx did not show any protective abilities against challenge with strain 733913.

Challenge with JEV strains 641686 and 691004

Though none of the MAbs given either 1 day prior or 2 days after the challenge with either of the two JEV group II strains protected the mice (mortality MR 20/20), an increase in mice survival was noticed ranging from half a day to almost three days with some of the MAbs as compared to the controls (AST 5.3–5.4 days) (Table 1 and 2). The pronounced effect was observed by the MAbs NHs-1 (AST 8.35 days) and Hx-3 (AST 7.65 days) given to mice 1 day before challenge with JEV strain 641686. In contrast, MAbs Hx-1 and Hx-2 (AST 6.30 days for both) administered 1 day prior to the challenge with other JEV strain 691004 showed only a marginal increase in the mice survival. Interestingly, the mice given MAb NHx-3 2 days after the challenge with JEV strain 691004 showed slightly reduced survival (AST 4.9 days), whereas the mice administered the MAb 2 days after the challenge with JEV strain 641686 showed an increase in their survival (AST 7.45 days).

Challenge with WNV strain 68856

The Hx MAbs given 1 day prior or 2 days after the challenge protected mice against WNV lethal infection. The protection was highest with MAb Hx-2 (MR 6/20 30%, AST 16.15 days) and lowest with MAb Hx-5 (MR 15/20 75%, AST 9.5 days) as compared to the controls (MR 20/20 100%, AST 6.75–6.95 days) (Table 3). Interestingly, MAb Hs-3 given 2 days after the WNV challenge protected the mice (MR 9/20 45%, AST 15.5 days), whereas the same MAb failed to protect mice, when administered 1 day prior to the virus challenge (MR 20/20 100%, AST 7.4 days).

Table 2. Survival of mice treated with MAbs before or after challenge with JEV strain 691004

MAb (Ig subtype)	MAb given i.p. 1 day before challenge		MAb given i.p. 2 day after challenge	
	AST in days (mean ± S.D.)	Statistical significance P	AST in days (mean ± S.D.)	Statistical significance P
Hs-1 (IgG1)	5.40 ± 0.50	NS	5.05 ± 0.82	NS
Hs-3 (IgG2a)	5.75 ± 0.44	<0.05	5.45 ± 0.82	NS
Hx-1 (IgG2b)	6.30 ± 0.47	<0.01	5.50 ± 0.51	<0.01
Hx-2 (IgG2b)	6.30 ± 0.47	<0.01	5.40 ± 0.50	<0.01
Hx-3 (IgG2b)	6.00 ± 0.32	<0.01	5.55 ± 0.82	<0.01
Hx-5 (IgG2b)	5.80 ± 0.41	<0.05	5.25 ± 0.91	<0.05
NHs-1 (IgG1)	5.75 ± 0.44	<0.05	6.00 ± 0.32	<0.05
NHx-3 (IgG2b)	5.80 ± 0.41	<0.05	4.90 ± 0.72	<0.05
SP2/0 ascites	5.40 ± 0.50	NS	5.95 ± 0.82	NS
CHICKV MAb				

NS, Hs, Hx, NHs, NHx – for explanation see Table 1.

Table 3. MR and AST in mice treated with MAbs before or after challenge with WNV

MAb	MAb given i.p. 1 day before challenge				MAb given i.p. 2 days after challenge			
	MR	Statistical significance X2, P	AST in days (mean ±S.D.)	Statistical significance P	MR	Statistical significance X2, P	AST in days (mean ±S.D.)	Statistical significance P
Hs-3	20/20 (100%)	NS	7.40 ± 4.02	NS	9/20 (45%)	X2 = 12.54 P <0.01	15.50 ± 6.00	<0.01
Hx-1	11/20 (55%)	X2 = 9.18 P <0.01	13.00 ± 7.07	NS	12/20 (60%)	X2 = 7.66 P <0.01	12.70 ± 6.57	<0.05
Hx-2	6/20 (30%)	X2 = 18.57 P <0.01	16.05 ± 6.52	<0.01	20/20 (100%)		6.75 ± 1.12	NS
Hx-5	15/20 (75%)	X2 = 3.66 NS	9.50 ± 6.26	NS	13/20 (65%)	X2 = 6.23 P <0.01	10.55 ± 7.13	NS
SP2/0 ascites CHICKV MAb	20/20 (100%)		6.75 ± 1.02		20/20 (100%)		6.95 ± 1.05	

NS, Hs, Hx – for explanation see Table 1.

Challenge with DENV-2 strain P23085

The Hx MAbs given 1 day prior to the challenge protected mice against DENV-2 lethal infection. The protection was highest with MAbs Hx-1 (MR 13/20 65%, AST 16.50 days) and Hx-5 (MR 13/20 65%, AST 15.8 days) as compared to the controls (MR 20/20 100%, AST 13.5 to 14.1 days) (Table 4). Interestingly, MAb Hs-3 given 2 days after the DENV-2 challenge protected the mice (MR 14/20 70%, AST 15.35 days), whereas the same MAb administered 1 day prior to the virus challenge failed to protect mice (MR 20/20 100%, AST 13.75 days).

Discussion

gE is a major flaviviral antigen that binds to the cellular receptors, mediates cell membrane fusion, and contains an array of epitopes that elicits production of virus neutralizing and non-neutralizing antibodies (Gould *et al*., 1986; Halstead *et al*., 1984; Mathews and Roehring, 1984; Kimura-Kuroda and Yasui, 1983; Heinz *et al*., 1983; Peiris *et al*., 1982). The protective efficacy of an anti-gE specific MAb is directly related to its ability to neutralize virus infectivity (Kimura-Kuroda and Yasui, 1988). The present study employing mouse model where MAbs in the form of AF were given 1 day prior or 2 days after the virus challenge is unique. Similarly both series of experiments match each other in assessing the antibody effectiveness against lethal virus infection under two different conditions when antibodies are already present in circulation or are administered after the establishment of virus infection in the brain, respectively (Gupta *et al*., 2003; Gould and Buckley, 1989).

In the present study, i.c. route of virus inoculation was employed that produced encephalitis in 100% of the adult Swiss mice. This is in contrast to the virus inoculation by peripheral routes that generally leads to the inapparent infection of the adult mice (Lad *et al*., 1993b; Gupta *et al*.,

Table 4. MR and AST in mice treated with MAbs before or after challenge with DENV-2

MAb	MAb given i.p. 1 day before challenge				MAb given i.p. 2 days after challenge			
	MR	Statistical significance X2, P	AST in days (mean ±S.D.)	Statistical significance P	MR	Statistical significance X2, P	AST in days (mean ±S.D.)	Statistical significance P
Hs-3	20/20 (100%)	NS	13.75 ± 2.55	NS	14/20 (70%)	X2 = 4.90 P <0.01	15.35 ± 3.15	NS
Hx-1	13/20 (65%)	X2 = 6.23 P <0.01	16.50 ± 2.84	<0.01	20/20 (100%)		13.40 ± 2.26	<0.05
Hx-2	15/20 (75%)	NS	14.10 ± 3.57	<0.01	20/20 (100%)		12.50 ± 1.85	<0.05
Hx-5	13/20 (65%)	X2 = 6.23 P <0.01	15.80 ± 3.19	NS	16/20 (80%)	NS	15.00 ± 3.26	NS
SP2/0 ascites CHICKV MAb	20/20 (100%)		13.50 ± 1.19		20/20 (100%)		14.10 ± 1.02	

NS, Hs, Hx – for explanation see Table 1.

1989; Kulkarni and Goverdhan, 1985). Group II of JEV strains lost some of the virus specific epitopes and at the same time they lost their ability to be neutralized with corresponding MAbs. On the other hand, group I of JEV strains maintained their neutralization ability by the corresponding MAbs (Gupta *et al*., 2006a, 2003, 2000; Ghosh *et al*., 1989). The differences in the survival of control mice administered with control SP2/0 or CHIKV MAb and challenged either with group I JEV strain 733913 or with group II JEV strains (641686, 691004) might be related to a high peripheral virulence shown by some of the latter JEV strains that displayed high neurovirulence after i.c. inoculation into adult mice (Gupta *et al*., 2006b; Lad *et al*., 2005). The HAI-positive flavivirus cross-reactive (Hx) MAbs that produced *in vitro* ADVE of JEV infection of cells did not show any effect *in vivo* (Cecilia and Ghosh, 1988). These MAbs were protective to some extent in mice against WNV and DENV infections. Initially, some protection by these MAbs against JEV group II strains was noticed, but all the mice eventually succumbed to the infection. However, some of these strains were neutralized at a low level with corresponding MAbs and therefore their *in vivo* effect using a lower virus dose remains to be tested. Also differences in the effect of the MAb NHx-3 on the JEV strains 641686 and 691004 in mice might be due to the alteration in the corresponding epitope. Still, applying of purified MAbs in defined proportions for evaluation of enhanced effects remains to be seen employing MAbs alone or in combinations with MAbs of the same group or other group.

Interestingly, HAI-positive JEV specific MAb Hs-3 administered 2 days after the virus challenge protected the mice against WNV or DENV-2 infection. The protection was more effective against WNV. The MAb Hs-3 grouped as JEV HAI-positive MAb corresponded to the epitope that partially overlapped the epitopes represented by two MAbs NHs-1 and NHx-2 that belonged to other domains (Cecilia *et al*., 1988). Therefore, the recognition of corresponding epitope by MAb Hs-3 on WNV and DENV might be due to the overlapping with other epitopes particularly those belonging to the flavivirus cross-reactive domain. It was shown that MAbs induced conformation-dependent changes in the viral epitopes (Gupta *et al*., 1999, 1992; Cepica *et al*., 1990). Therefore, the role of such changes in protection by the MAb Hs-3 against WNV and DENV infections in mice cannot be ruled out. Studies by Gould *et al*. (1986) employing neutralizing and non-neutralizing MAbs against Yellow fever virus (YFV) suggested that the ability of an antibody to protect the mice passively against the i.c. virus challenge depended on the virus neurovirulence. Also protection afforded by a non-neutralizing MAb against YFV was the indication of other factors involvement rather than neutralization alone that might be responsible for such an effect in mice (Gould *et al*., 1986).

Natural immunization is considered as an important factor limiting spread of the disease in an epidemic region. Japanese encephalitis incidence has shown to decrease with the increased presence of JEV antibodies in the immune population/group (Mohan Rao *et al*., 1983; Chakravorty *et al*., 1975). Studies carried out earlier on two way cross-protection in bonnet macaques (*Macaca radiata*) employing intranasal route of virus challenge revealed that immunization with JEV protected the animals against WNV infection, whereas WNV immunization reduced the disease severity produced by JEV inoculation (Goverdhan *et al*., 1992). Studies employing MAbs against gE of DENV or WNV against experimental JEV infections in mice might help in better understanding of the cross-protection phenomenon that might be occurring in some of the localities endemic for the three closely related flaviviruses.

Acknowledgements. Authors thank the Director of National Institute of Virology, Pune for providing the facilities, and Mrs. S.A. Sarthi and S. Argade for providing the MAbs.

References

Carey DE, Myers RM (1968): Japanese encephalitis studies in Vellore, South India. Part III. Neutralizing activity of human survey sera. *Indian J. Med. Res*. **56**, 1330–1339.

Cecilia D, Gadkari D, Kedarnath N, Ghosh SN (1988): Epitope mapping of Japanese encephalitis virus envelope protein using monoclonal antibodies against an Indian strain. *J. Gen. Virol.* **69**, 2741–2747.

Cecilia, D, Ghosh SN (1988): Antibody dependent plaque enhancement by monoclonal antibodies against Japanese encephalitis virus. *Indian J. Med. Res*. **67**, 521–523.

Cepica A, Yason C, Ralling G (1990): The use of ELISA for detection of antibody induced conformational change in viral protein and its intermolecular spread. *J. Virol. Methods* **28**, 1–14.

Chakravorty SK, Sarkar JK, Chakravorty MS, Mukerjee MK, Mukerjee KK, Das BC, Hati AK (1975): The first evidence of JE studied in India-virological studies. *Indian J. Med. Res*. **63**, 77–82.

George S, Gourie-Devi M, Rao JA, Prasad SR, Pavri KM (1984): Isolation of West Nile virus from the brains of children who died of encephalitis. *Bull. WHO* **62**, 879–882.

Ghosh SN, Sathe PS, Sarthi SA, Cecilia D, Dandawate CN, Athawale SS, Pant U (1989): Epitope analysis of Japanese encephalitis virus by monoclonal antibodies. *Indian J. Med. Res*. **89**, 368–375.

Gould EA, Buckley A, Barrett ADT, Cammack N (1986): Neutralizing (54 K) and non-neutralizing (54 K and 48 K) monoclonal antibodies against structural and non-structural yellow fever virus proteins confer immunity in mice. *J. Gen. Virol*. **67**, 591–595.

Gould EA, Buckley A (1989): Antibody-dependent enhancement of yellow fever and Japanese encephalitis virus neuro-virulence. *J. Gen. Virol*. **70**, 1605–1608.

Goverdhan MK, Kulkarni AB, Gupta AK, Tupe CD, Rodrigues JJ (1992): Two-way cross protection between West Nile and Japanese encephalitis viruses in Bonnet macaques. *Acta Virol.* **36**, 277–283.

Gupta AK, Ayachit VL, Lad VJ, Rodrigues JJ (1989): Protective role of immunity in experimental Japanese encephalitis virus Infections in mice. *Indian J. Virol*. **5**, 1–8.

Gupta AK, Bhattacharya S, Lad VJ, Sarthi SA, Kutubddin M, Ghosh SN, Banerjee K (1992): Monoclonal antibody to Japanese encephalitis virus cross-reacting with histones present in the cell nuclei. *Acta Virol*. **36**, 401–411.

Gupta AK, Lad VJ, Koshy AA (2003): Protection of mice against experimental Japanese encephalitis virus infection by neutralizing anti-glycoprotein 'E 'monoclonal antibodies. *Acta Virol*. **47**, 141–145.

Gupta AK, Lad VJ, Koshy AA (2006a): Protection against infection of group II Japanese encephalitis virus strains in mice. *Indian J. Virol.* **17**, 9–14.

Gupta AK, Lad VJ, Koshy AA, Gadkari DA (2000): Loss of virus specific epitopes on JE virus glycoprotein by acetone treatment. *Indian J. Med. Res*. **112**, 113–120.

Gupta AK, Lad VJ, Sarthi SA, Koshy AA, Gadkari DA (1999): An IgM monoclonal antibody to JE virus recognizing a cross-reacting epitope on nuclear histones. *Indian J. Med. Res*. **110**, 149–154.

Gupta AK, Koshy AA, Damle RG, Gadkari DA (2006b): An Indian strain of Japanese encephalitis virus showing high peripheral virulence in adult Swiss mice. *Indian J. Virol*. **17**, 8–13.

Halstead SB, Venkateshan CN, Gentry MK, Larsen LK (1984): Heterogeneity of infection enhancement of dengue 2 strains by monoclonal antibodies. *J. Immunol.* **132**, 1529–1532.

Hanna JN, Ritchie SA, Philips DA, Shield J, Bailey MC, Mackenzie JS, Poidinger M, McCall BJ, Millis PJ (1996): An outbreak of Japanese encephalitis in the Torres Strait, Australia, 1995. *Med. J. Aust.* **165**, 256–260.

Hanna JN, Ritchie SA, Philips DA, Lee JM, Hills SL, van den Hurk AF, Pyke T, Johnson CA, Mackenzie JS (1999): Japanese encephalitis North Queens land, Australia, 1998. *Med. J. Aust*. **170**, 533–566.

Heinz FX, Berger R, Tuma W, Kunz C (1983): A topological and functional model of epitopes on the structural glycoprotein of Tick-borne encephalitis virus defined by monoclonal antibodies. *Virology* **126**, 525–537.

Kedarnath N, Dayaraj C, Sathe PS, Gadkari DA, Dandawate CN, Goverdhan MK, Ghosh SN (1986): Monoclonal antibodies against Japanese encephalitis virus. *Indian J. Med. Res.* **84**, 125–133.

Kimura-Kuroda J, Yasui K (1983): Topographical analysis of antigenic determinants on envelope glycoprotein V3 (E) of Japanese encephalitis virus using monoclonal antibodies. *J. Virol.* **45**, 124–132.

Kimura-Kuroda J, Yasui K (1988): Protection of mice against Japanese encephalitis virus by passive administration with monoclonal antibodies. *J. Immunol.* **141**, 3606–3610.

Kulkarni AB, Goverdhan MK (1985): Age related susceptibility of Swiss mice to Japanese encephalitis virus by intraperitoneal route. *Biol. Mem*. **10**, 141–145.

Lad VJ, Gupta AK, Ayachit VL, Dandawate CN (2005): Peripheral pathogenicity of a Chinese strain (Beijing P3) of JE virus in immunodeficient and immunocompetent mice. *Indian J. Virol*. **112**, 113–120.

Lad VJ, Gupta AK, Ghosh SN, Banerjee K (1993a): Immunofluorescent studies on the replication of some arboviruses in nucleated and enucleated cells. *Acta Virol*. **37**, 79–82.

Lad VJ, Gupta AK, Goverdhan MK, Ayachit VL, Rodrigues JJ, Hungund LV (1993b): Susceptibilty of BL 6 nude (congenitally athymic) mice to Japanese encephalitis virus by the peripheral route. *Acta Virol*. **37**, 232–240.

Mathews JH, Roehring JT (1984): Elucidation of the topography and determination of the protective epitopes on the E glycoprotein of Saint Louis encephalitis virus by passive transfer with monoclonal antibodies. *J. Immunol*. **132**, 1533–1537.

Mohan Rao CVR, Prasad SR, Rodrigues JJ, Sharma NGK, Shaikh BH, Pavri KM (1983): The first laboratory proven outbreak of Japanese B encephalitis in Goa. *Indian J. Med. Res*. **78**, 745–750.

Monath TP (1985): Flaviviruses. In Fields BN (Ed.): *Virology.* New York, Raven Press, pp. 955–1004.

Monath TP, Heinz FX (1996): Flaviviruses. In Fields BN, Koipe DM, Howley PM (Ed.): *Field's Virology. Field's Fundamental Virology*. 3rd ed. Philadelphia, Lippincott-Raven, pp. 961–1034.

Peiris JSM, Porterfield JS, Roehring JT (1982): Monoclonal antibodies against the flavivirus West Nile virus. *J. Gen. Virol*. **58**, 283–289.

Rodrigues FM (1984): Epidemiology of Japanese encephalitis in India: A brief overview. In *Proc. Natn. Conf. Jap. Enceph.*, Indian Council of Medical Research, November 1982, New Delhi, India, pp. 1–9.

Roehring JT (1986): The use of monoclonal antibodies in studies of the structural proteins of togaviruses and flaviviruses. 111. Flaviviruses. In Schlesinger S, Schlesinger MJ (Ed.): *The Togaviridae and Flaviviridae*. Plenum Press, pp. 265–272.

Vaughn DW, Hoke CH (1992): The epidemiology of Japanese encephalitis: prospects for prevention. *Epidemiol. Rev.* **14**, 197–221.