PROTECTION OF MICE AGAINST EXPERIMENTAL JAPANESE ENCEPHALITIS VIRUS INFECTIONS BY NEUTRALIZING ANTI-GLYCOPROTEIN E MONOCLONAL ANTIBODIES

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

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

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Summary. – Neutralizing monoclonal antibodies (MAbs) to glycoprotein E (gpE) of Japanese encephalitis (JE) virus given intraperitoneally (i.p.) (0.1 ml of immune ascitic fluid (AF) diluted 1:10 per mouse) to about 4-week-old Swiss mice 1 day prior or 2 days after the virus challenge (100 LD_{50} of JE virus administered intracerebrally (i.c.)) resulted in a decreased mortality along with an increased survival of the animals as demonstrated by the HAI-positive virus-specific (Hs) MAbs. The protective effect produced by four Hs MAbs was maximum when given 1 day prior the virus challenge, while other, namely HAI-positive flavivirus cross-reactive (Hx) and HAI-negative virus-specific (NHs) MAbs did not produce any effect. Interestingly, one of the two NHs MAbs, namely NHs-1 showed a reduced survival of mice given the MAb 2 days after the virus challenge. Administration of combinations of two or more Hs MAbs may be recommended due to their possible enhanced protection against JE virus infections in mice.

Key words: Japanese encephalitis virus; monoclonal antibodies; protection; Swiss mice

Introduction

JE virus is one of the mosquito-borne flaviviruses which often causes epidemics of viral encephalitis in South-East Asian and Western Pacific regions (Rodrigues, 1984; Vaughn and Hoke, 1992; Monath and Heinz, 1996). However, the situation in South-East Asia is complicated further due to overlapping epidemics of JE and Dengue (DEN) viruses as well as sporadic cases caused by West Nile (WN) virus in some of the affected areas (George *et al*., 1984). These three antigenically-related viruses have posed serious hazard to public health and thus complicated to a great extent both vaccination and host immunologic responses.

Epitope analysis of the structural envelope (E) protein of flaviviruses provides the most fundamental information for development of an effective vaccine along with elucidating some aspects of pathogenesis or the mechanism of infection (Kimura-Kuroda and Yasui, 1988). Important biological functions of hemagglutination (HA) and neutralization are attributable to this protein, as well as antigenic specificities that range from specific to cross-reactive (Peiris *et al*., 1982; Heinz *et al*., 1983; Kimura-Kuroda and Yasui, 1983; Halstead *et al*., 1984; Mathews and Roehrig, 1984; Gould *et al*., 1986).

E-mail: Guptaak2002@yahoo.co.in, icmrniv@icmrniv.ren.nic.in, nivicl@pn3.vsnl.net.in; fax: +9020-6122669, +9020-6123679.

Abbreviations: ADVE = antibody-dependent virus enhancement; AF = ascitic fluid; AST = average survival time; BB = blood brain; DEN = Dengue; FCS = fetal calf serum; gp = glycoprotein; HA = hemagglutination; HAI = hemagglutination-inhibition; Hs = HAI-positive virus-specific; Hx = HAI-positive flavivirus cross-reactive; i.c. = intracerebral(ly); i.p. = intraperitoneal(ly); JE = Japanese encephalitis; MAb = monoclonal antibody; MR = mortality ratio; NHA = HAI-negative autoreactive; NHs = HAI-negative virus-specific; NHx = HAI-negative flavivirus cross-reactive; p.i. = post infection; WN = West Nile; YF = yellow fever

Mapping of gpE for antigenic epitopes on JE virus Indian strain 733913 employing MAbs raised earlier in our laboratory (Kedarnath *et al*., 1986) have shown the existence of five domains represented by Hs, NHs, Hx, HAI-negative flavivirus cross-reactive (NHx) and HAI-negative autoreactive (NHA) MAbs (Cecilia *et al*., 1988). The MAbs representing Hs and NHs epitopes are neutralizing, while those belonging to the Hx domain depict the antibody-dependent virus enhancement (ADVE) *in vitro* in addition to neutralization (Cecilia and Ghosh, 1988). NHA MAbs probably induce autoimmunity due to cross-reactions with nuclear histones (Gupta *et al.*, 1992, 1999).

This communication deals with details of the *in vivo* protection if any, shown by neutralizing Hs, NHs and some of the Hx MAbs against experimental virus challenge with the JE virus Indian strain 733913 in Swiss mice.

Materials and Methods

Virus strain and mice. The JE virus strain 733913 isolated earlier from the brain of a fatal human JE case from Bankura, West Bengal, India in 1973 (Ghosh *et al*., 1989) was employed in the study. It was maintained at the NIV by intracerebral (i.c.) passaging in 2-day-old suckling mice and was titrated both in 3 to 4-week-old and 2-day-old infant mice by i.c. and intraperitoneal (i.p.) routes. The mice employed in the experiments have been initially (1956–1960) supplied by the Rockefeller Foundation Virus Laboratories, New York, USA, but later they have been reared and maintained by random breeding (the NIV stock) at the Animal House facility of the NIV. The mice of about 4 weeks of age were used in the study as approved by the Institutional Animal Ethics Committee for the animal maintenance and the experimental work at NIV.

MAbs against gpE of JE virus strain 733913 prepared earlier in our laboratory and characterized initially for their reactivities with JE, WN and DEN-2 viruses (Kedarnath *et al*., 1986) have been employed later for epitope mapping of gpE of JE virus. They were grouped as Hs (4 MAbs), NHs (2 MAbs), Hx (5 MAbs), NHx (3 MAbs) and NHA (2 MAbs) (Cecilia *et al*., 1988). All the four Hs and two NHs neutralizing MAbs, but only two of the five Hx MAbs, which were neutralizing in addition to producing ADVE *in vitro* (Cecilia and Ghosh, 1988), were included in the study. The hybrid clones were maintained in the Dulbecco's Minimum Essential Medium (MEM) with 10% of fetal calf serum (both from Gibco). Ascitic fluids (AFs) obtained from ascites produced in pristane-primed BALB/c mice after inoculation of hybrid cells were used. The AF obtained from the mice inoculated with SP2/o cells served as a negative control.

The experiment. Groups of about 4-week-old mice were inoculated i.c. with 100 LD_{50} of the virus. At 2 days p.i. the mice were injected i.p. with 0.1 ml of immune AF (diluted 1:10) containing the tested MAb. The mice were observed for 21 days and the mortality ratio (MR) and average survival time (AST) were calculated and compared with those of control mice. The mice inoculated similarly with JE virus and given 0.1 ml of AF containing SP2/o cells served as controls. In another series of experiments about 4-week-old mice were administered i.p. 0.1 ml of the immune or control AF (SP2/o cells) (diluted 1:10) 1 day prior the virus challenge (100 LD_{50} i.c). MR and AST were calculated similarly as above.

Statistical significance of differences from control values was evaluated by χ^2 test. P values ≤ 0.05 were considered significant.

Results and Discussion

The Hs MAbs tested in the present study protected the mice against virus challenge; the protection was maximum when MAbs were given 1 day before the challenge (Tables 1 and 2). Of the four Hs MAbs the protection was maximum with MAbs Hs-4 (MR 30%, AST 15 days) and Hs-1 (MR 47%, AST 12.6 days) as compared to that of the controls (MR 98%, AST 6.9 days), but it was lesser with MAbs Hs-2 (MR 63%, AST 11.44 days) and Hs-3 (MR 60%, AST 11.26 days). The protection was significantly reduced when the Hs MAbs were given 2 days after the virus challenge; MAbs Hs-1 (MR 70%, AST 9.79 days) and Hs-2 (MR 80%, AST 8.1 days) exerted a little protection, but MAbs Hs-3 (MR 94%, AST 7.6 days) and Hs-4 (MR 95%, AST 6.83 days) did not have any effect.

The gpE is a major flaviviral antigen that binds cellular receptors, mediates cell membrane fusion and contains an array of epitopes that elicit virus-neutralizing and non-neutralizing antibodies. The protective efficacy of an anti-gpE-specific MAb is thus directly related to its ability to neutralize the virus infectivity (Kimura-Kuroda and Yasui, 1988). The present study employing mouse model in which MAbs (immune AFs) were given 1 day prior to or 2 days after the virus challenge, is unique as both series of experiments complement each other in assessing the antibody effectiveness against lethal virus challenge under different conditions when antibodies are already present in circulation or antibodies are given after the establishment of virus infection in the brain (Gould and Buckley, 1989), respectively.

In the present study, the i.c. route of virus inoculation was employed producing encephalitis in 97% and more of adult Swiss mice. This is in contrast to the virus inoculation by peripheral routes generally leading to inaparent infections in adult Swiss mice (Kulkarni and Goverdhan, 1985; Gupta *et al*., 1989; Lad *et al*., 1993). The antibodies especially the Hs group of MAbs administered i.p. are able to protect adult Swiss mice against the virus challenge given by i.c. route even though it is well known that IgGs are unable to cross the blood-brain barrier (BB) (Bradbury, 1985) to reach the brain wherein the virus is localized. The JE virus replication is known to induce changes/damage to the BB barrier (Gupta *et al*., 1989; Lad *et al*., 2000) and is in the process permitting

Table 1. Mortality in mice administered MAbs prior or after the virus challenge

MAb	Ig subtype	JE virus challenge by i.c. route			
		MAb given i.p. 1 day prior to the challenge		MAb given i.p. 2 days after the challenge	
		MR	Statistical significance (χ^2, P)	MR	Statistical significance (χ^2, P)
Hs (HAI-positive JE virus-specific) MAbs					
Hs-1	IgG1	9/19 (47%)	S (23.85, P <0.001)	(14/20 (70%)	S (8.44, P <0.005)
Hs-2	IgG2a	12/19 (63%)	S (14.64, P <0.001)	16/20 (80%)	S (4.53, P <0.05)
Hs-3	IgG2a	12/20 (60%)[a]	S (16.57, P <0.001)	17/18 (94%)[b]	NS
Hs-4	IgG2a	6/20 (30%)[c]	S (35.83, P <0.001)	18/19 (95%)[d]	NS
Hx (HAI-positive flavivirus cross-reactive) MAbs					
Hx-1	IgG2b	19/20 (95%)	NS	19/20 (95%)	NS
Hx-3	IgG2b	17/20 (85%)	NS	20/20 (100%)	NS
NHs (HAI-negative JE virus-specific) MAbs					
NHs-1	IgG1	18/20 (90%)	NS	19/19 (100%)	NS
NHs-2	IgG1	20/20 (100%)	NS	18/20 (90%)	NS
Negative control					
(SP2/o)		44/45 (98%)		34/35 (97%)	

MR = mortality ratio, S = significant, NS = non-significant.

Significance of the differences between a and b: $\chi^2 = 6.22$, $P \leq 0.05$, S and between c and d: $\chi^2 = 17.25$, $P \leq 0.001$, S.

Table 2. AST in mice administered MAbs prior or after the virus challenge

MAb	Ig subtype	JE virus challenge by i.c. route			
		MAb given i.p. 1 day prior to the challenge		MAb given i.p. 2 days after the challenge	
		AST in days (mean ± SD)	Statistical significance (P)	AST in days (mean ± SD)	Statistical significance (P)
Hs (HAI-positive JE virus-specific MAbs					
Hs-1	IgG1	12.60 ± 0.21	S ($P \leq 0.001$)	9.79 ± 0.21	NS
Hs-2	IgG2a	11.44 ± 0.20[a]	S ($P \leq 0.001$)	8.10 ± 0.20[b]	NS
Hs-3	IgG2a	11.26 ± 0.21[c]	S ($P \leq 0.001$)	7.60 ± 0.16[d]	NS
Hs-4	IgG2a	15.01 ± 0.19[e]	S ($P \leq 0.001$)	6.83 ± 0.16[f]	NS
Hx (HAI-positive flavivirus cross-reactive) MAbs					
Hx-1	IgG2b	7.77 ± 0.15	NS	6.82 ±0.15	NS
Hx-3	IgG2b	7.27 ± 0.21	NS	6.46 ± 0.10	NS
NHs (HAI-negative JE virus-specific) MAbs					
NHs-1	IgG1	7.37 ± 0.18	NS	6.00 ± 0.12	NS
NHs-2	IgG1	6.82 ± 0.06	NS	7.00 ± 0.16	NS
Negative control					
(SP2/o)		6.90 ± 0.09		7.10 ± 0.10	

AST = average survival time, S = significant, NS = non-significant.

Significance of the differences between a and b: $P \leq 0.05$, S, between c and d : $P \leq 0.01$, S; and between e and f: $P \leq 0.001$, S.

the antibody to cross and interact/neutralize the virus inside the brain, which might be thus responsible for the protection of experimental animals. However, studies by Gould *et al.* (1986) employing neutralizing and non-neutralizing MAbs against yellow fever (YF) virus suggest that the ability of an antibody to protect the mice passively against the i.c. virus challenge depends on the neurovirulence of virus for mice. Also the protection afforded by a non-neutralizing MAb against YF virus is indicative of other factors involvement rather than neutralization alone which might be responsible for such an effect (Gould *et al*., 1986).

Since each MAb recognizes specifically a single epitope on gpE and is responsible for neutralization of JE virions (Peiris *et al*., 1982; Kimura-Kuroda and Yasui, 1983), a combination of two or more Hs MAbs may have a maximum protection against JE virus infections in mice due to synergism. Preliminary experiments employing a combination of the four Hs MAbs showed better protective effects in mice as compared to individual Hs MAbs (data not shown). However, applying purified immune proteins (MAbs) in defined proportions for evaluating their enhanced protective effects, if any, remains to be done.

The Hx and NHs MAbs did not show any protection when given 1 day prior to or 2 days after the challenge. Interestingly, one of the NHs MAbs, namely NHs-1 given 2 days after the challenge showed some AST reduction, though statistically insignificant.

The latter effect may indicate modification of viral biological activity leading to an enhanced virus virulence (Gould and Buckley, 1989) as similar titers were recorded in the brains of a few mice harvested just prior to death from both the controls and experimental animals (data not shown). Such an effect of the MAb on the survival of mice was not observed when it was already present in circulation prior to the virus challenge. As MAbs have been found to induce conformation-dependent changes in viral epitopes (Cepica *et al*., 1990; Gupta *et al*., 1992, 1999), the role of such changes leading to enhanced viral virulence cannot be ruled out However, conformational alterations of viral proteins induced by the antibody attachment might also reduce the efficiency of viral infections as indicated for Sindbis (Clegg *et al*., 1983) and Yellow Fever virus (Gould *et al*., 1986). Further studies of this aspect employing a larger number of animals will be needed to understand the significance of this finding.

Also Hx MAbs have been earlier shown *in vitro* to enhance the virus replication (ADVE) in cells (Cecilia and Ghosh, 1988). However, no such effect seemed to have occurred *in vivo* as the two Hx MAbs did not lower the AST in mice upon the virus challenge. Studies are being carried out to elucidate the role of NHs and Hx epitopes in experimental infections with different JE virus strains.

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References

Bradbury MWB (1985): The blood-brain barrier. Transport across the cerebral endothelium. *Circulation Res.* **57**, 213–222.

Cecilia D, Gadkari DA, 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.* **87**, 521–525.

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

Clegg CS, Chanas AC, Gould EA (1983): Conformational changes in Sindbis virus E1 glycoprotein induced by monoclonal antibody binding. *J. Gen. Virol*. **64**, 1121–1126.

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 (1989): Epitope analysis of strains 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 neurovirulence. *J. Gen. Virol.* **70**, 1605–1608.

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

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.

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, Goverdhan MK, Ayachit VL, Rodrigues JJ, Hungund LV (1993): Susceptibility of BL6 nude (congenitally athymic) mice to Japanese encephalitis virus by the peripheral route. *Acta Virol.* **37**, 232–240.

Lad VJ, Gupta AK, Ayachit VL, Dandawate CN (2000): Peripheral pathogenicity of a Chinese strain (Beijing P3) of JE virus in immunodeficient and immunocompetent mice. *Indian J. Virol.* **112**, 113–120.

Mathews JH, Roehrig 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.

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

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

Rodrigues FM (1984): Epidemiology of Japanese encephalitis in India: a brief overview. *National Conference on Japanese Encephalitis*. Indian Council of Medical Research, New Delhi.

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