Protection of mice against Japanese encephalitis virus group II strain infections by combinations of monoclonal antibodies to different antigenic domains on glycoprotein E

Ashok Kumar Gupta, Attiyaril Abraham Koshy, Vaibhavi Jawahar Lad
National Institute of Virology, Pune, India

Abstract

A combination of at least three hemagglutination-inhibition-positive (HAI) and virus-specific (Hs) monoclonal antibodies (MAbs) to glycoprotein E (gpE) of Japanese encephalitis virus (JEV) fully protected (100%) mice against JEV strain 733913 infections (group 1). However, these representative epitopes are reported to have been lost on JEV group II strains. In the present study, therefore, the protective effect of various combinations of anti-gpE MAbs representing antigenic epitopes other than Hs was studied on mice infections with JEV group II strains: JEV strains 641686 and 691004. MAbs used in the protective experiments were characterized as HAI-negative virus-specific (NHs) and HAI-positive flavivirus cross-reactive (Hx). Additionally, one of the Hs MAbs (MAb Hs-3) was included in the experiments. Mice were first administered single MAbs or their combinations intraperitoneally and 24 h later, infected with the virus intracerebrally. Protection rates of 70-75% were obtained with a combination of four MAbs: MABs NHs-1, Hx-1, Hx-3 and Hs-3. However, protection rates of only 20-40% were obtained with three MAbs but none was observed with single or two MABs. There was, however, a substantial increase in mice survival. The protective effect of several combinations of anti-gpE MAbs representing different antigenic epitopes might be due to the enhancement of binding within the same group and also between different MAb groups. The present results indicate that NHs and Hx epitopes should be incorporated with three Hs epitopes in a JEV vaccine that would have an added advantage, particularly in the flaviviral endemic areas with JEV strain variations.

Introduction

Japanese encephalitis virus (JEV, the genus Flavivirus and family Flaviviridae), belongs to a mosquito-borne flavivirus group consisting of some 66 antigenically-related viruses. The virus has gained considerable importance as a human pathogen with an increase in frequency of epidemics of viral encephalitis as recorded throughout South-East Asia and Western Pacific regions. Mortality as high as 40% was recorded in some of the Japanese encephalitis (JE) affected areas. Furthermore, many survivors face some neurological problems and complications. Since 1995, the disease has also been shown to emerge in non-Asian regions such as Northern Australia. The situation in South-East Asia, however, is further complicated by overlapping epidemics of JEV and dengue virus (DENV) as well as sporadic cases caused by West Nile virus (WNV). These all pose a serious hazard to public health. This has greatly complicated both vaccination and host immunological responses. The glycoprotein E (gpE) of flaviviruses contains most of the antigenic epitopes which induce various biological functions including hemagglutination and neutralization, and show antigenic reactivity that ranges from specific to cross-reactive. Mapping of gpE for antigenic epitopes on JEV strain 733913 (group 1 of Indian origin) employing monoclonal antibodies (MAbs) raised earlier in our laboratory and characterized initially for reactivity with JEV, DENV-2 and WNV were used in this study to determine the protective effect of combinations of two or more MAbs on mice infections with JEV group II strains.

Materials and Methods

Mice and viruses

The details of the Swiss mice are given elsewhere. Randomly bred 4-week old mice were used in this study as approved by the Institutional Animal Ethics Committee at the National Institute of Virology, Pune, India. Two JEV strains (641686 of Indian origin and 691004 of Sri Lankan origin) were characterized as group II JEV strains on the basis of loss of Hs epitope functional activity. Furthermore, 21 JEV strains analyzed for the antigenic epitopes employing these MAbs revealed 15 strains with complete Hs epitope functional activity and neutralized by the Hs MAbs. These were placed in group 1. The remaining 6 JEV strains that have lost their neutralization ability with most of the Hs MAbs and neutralized by the MAbs representing NHs and Hx epitopes were grouped as group II strains.

Also in protection experiments in mice with four Hs MAbs administered alone, 45-65% protection against JEV strain 733913 was noticed which was enhanced to 85-90% and 100% protection when the MAbs were given in combinations of two or three Hs, respectively. In contrast, no protection with Hs and another group of MAbs against the JEV group II strain infections was noticed except an increase in mice survival with the NHs and Hx MAbs, and to some extent also with MABs Hs-3. However, their additive or enhanced effect has not yet been examined.

The present study was, therefore, undertaken to determine the protective effect of combinations of two or more MAbs on mice infections with JEV group II strains (strains 641686 and 691004).
Results

Challenge with Japanese encephalitis virus strain 733913

The effect of MAbs administered singly on the homologous JEV strain 733913 has already been examined in mice and the results were similar to those obtained in the present study.16,20 Those mice challenged with homologous virus served as positive control to the mice challenged with heterologous viruses. The results are invariably similar and, therefore, are not presented.

Table 1. Mortality of Japanese encephalitis virus (strain 641686)-infected mice passively given single monoclonal antibodies to Japanese encephalitis virus glycoprotein E and their combinations

| Mice administered | Mortality (%) |
|-------------------|--------------|
| No MAb (control) | Normal IgG | 20/20 (100) |
| Single MAb* | NHs-1 | 20/20 (100) |
| | Hs-3 | 20/20 (100) |
| | Hx-1 | 20/20 (100) |
| | Hx-3 | 20/20 (100) |
| Combinations of two MAbs° | Hs-3+Hx-1 | 20/20 (100) |
| | Hs-3+Hx-3 | 20/20 (100) |
| | Hs-3+Hx-3 | 20/20 (100) |
| | Hx-1+Hx-3 | 20/20 (100) |
| | Hx-1+NHs-1 | 20/20 (100) |
| | Hx-3+NHs-1 | 20/20 (100) |
| Combinations of three MAbs° | Hs-3+Hx-1+Hx-3 | 12/20 (60) |
| | Hs-3+Hx-1+NHs-1 | 16/20 (80) |
| | Hx-1+Hx-3+NHs-1 | 12/20 (60) |
| | Hx-3+Hx-3+NHs-1 | 15/20 (75) |
| Combinations of four MAbs | Hs-3+Hx-1+Hx-3+NHs-1 | 6/20 (30) |

Table 2. Mortality of Japanese encephalitis virus (strain 691004)-infected mice passively given single monoclonal antibodies to Japanese encephalitis virus glycoprotein E and their combinations.

| Mice administered | Mortality (%) |
|-------------------|--------------|
| No MAb (control) | Normal IgG | 20/20 (100) |
| Single MAb* | NHs-1 | 20/20 (100) |
| | Hs-3 | 20/20 (100) |
| | Hx-1 | 20/20 (100) |
| | Hx-3 | 20/20 (100) |
| Combinations of two MAbs° | Hs-3+Hx-1 | 20/20 (100) |
| | Hs-3+Hx-3 | 20/20 (100) |
| | Hs-3+Hx-3 | 20/20 (100) |
| | Hx-1+Hx-3 | 20/20 (100) |
| | Hx-1+NHs-1 | 20/20 (100) |
| | Hx-3+NHs-1 | 20/20 (100) |
| Combinations of three MAbs° | Hs-3+Hx-1+Hx-3 | 13/20 (65) |
| | Hs-3+Hx-1+NHs-1 | 14/20 (70) |
| | Hx-1+Hx-3+NHs-1 | 13/20 (65) |
| | Hx-3+Hx-3+NHs-1 | 16/20 (80) |
| Combinations of four MAbs | Hs-3+Hx-1+Hx-3+NHs-1 | 5/20 (25) |

Mice administered Mortality (%) | No MAb (control) | Normal IgG | 20/20 (100) |
| Single MAb* | NHs-1 | 20/20 (100) |
| | Hs-3 | 20/20 (100) |
| | Hx-1 | 20/20 (100) |
| | Hx-3 | 20/20 (100) |
| Combinations of two MAbs° | Hs-3+Hx-1 | 20/20 (100) |
| | Hs-3+Hx-3 | 20/20 (100) |
| | Hs-3+Hx-3 | 20/20 (100) |
| | Hx-1+Hx-3 | 20/20 (100) |
| | Hx-1+NHs-1 | 20/20 (100) |
| | Hx-3+NHs-1 | 20/20 (100) |
| Combinations of three MAbs° | Hs-3+Hx-1+Hx-3 | 12/20 (60) |
| | Hs-3+Hx-1+NHs-1 | 16/20 (80) |
| | Hx-1+Hx-3+NHs-1 | 12/20 (60) |
| | Hx-3+Hx-3+NHs-1 | 15/20 (75) |
| Combinations of four MAbs | Hs-3+Hx-1+Hx-3+NHs-1 | 6/20 (30) |

MAbs, monoclonal antibodies; IgG, immunoglobulin G; Hs, HAI positive virus-specific; NHs, HAI negative virus-specific; Hx, HAI positive flavivirus cross-reactive. Individual MAbs were administered in 100 μg per mouse. In addition, 100 μg of normal IgG per mouse; 200 μg of normal IgG per mouse and 100 μg of normal IgG per mouse were given into mouse groups °, ° and °, respectively.

Protection experiments

The experiments were carried out as described elsewhere.16,17,20 In brief, 4-week old Swiss mice were divided into groups of 20 animals and administered a combinations of 4 MAbs intraperitoneally (total 0.4 mL) with 100 μg of purified IgGs of each MAb per mouse. For 2 or 3 MAb combinations, 200 μg or 100 μg of normal IgGs were injected along with the MAbs, respectively. Twenty-four hours later, the mice were challenged i.c. with 100 LD₅₀ of the virus. Groups of mice were administered singly with MAbs NHs-1, Hx-1, Hx-3 or MAb Hs-3, or with JEV antiserum (100 μg IgGs per mouse) along with the injection of 300 μg of normal IgGs to obtain the same level of administered IgG in each animal. Also, an additional group of mice were administered normal IgG (300-400 μg/mouse) or normal mouse serum (diluted 1:10) in each experimental group as a negative control. The mice were observed for 21 days and the mortality expressed as number of mice died/ total number of the mice tested (%) was compared with that of control mice. Average survival time (AST) of the dead mice in days was further determined as described previously.16,20 However, those surviving the virus challenge till days 21 were excluded from the analysis.

The effect of MAbs administered singly on the homologous JEV strain 733913 has already been examined in mice and the results were similar to those obtained in the present study.16,20 Those mice challenged with homologous virus served as positive control to the mice challenged with heterologous viruses. The results are invariably similar and, therefore, are not presented.

Challenge with Japanese encephalitis virus, strains 641686 and 691004

The mortality of mice treated with single Mab or MAbs in various combinations is presented in Tables 1 and 2. Any of the 4 MAbs IgGs or even combinations of 2 MAbs did not protect the mice since 100% (20 of 20) mortality like in the controls (20 of 20; 100%) was recorded. In contrast, 60-80% and 25-30% mortality was recorded with combinations of 3 or 4 MAbs, respectively. Initially, the mice administered...
100 μg of purified JEV antiserum IgG were protected 100% against JEV group I and group II strains; therefore, the same dose of the purified MAbs IgGs was used in the protection experiment. Although no statistical comparison with the controls (AST 5.4-5.5 days) was made, an increase in survival of the mice was noticed ranging from half a day to almost three days with the single MAbs. The pronounced effect was observed by the MAbs NHs-1 (AST 8.3 days) and Hx-3 (AST 7.7 days) against JEV strain 641686, while the increase was only marginal with MAbs Hx-1 (AST 6.2 days) and Hx-3 (AST 6.1 days) against JEV strain 691004. Similarly, with 2 or 3 MAB combinations, an increase in survival of the mice was noticed with either of the 2 JEV strains ranging from approximately 4-7 days (AST 8.3-12.3 days) and approximately seven to almost ten days (AST 12.8-15.3 days), respectively. In contrast, with 4 MAB combinations, the survival of mice was enhanced by approximately 11 to over 12 days with both of the JEV strains: 641686 (AST 16.3 days) and 691004 (AST 17.9 days).

The mortality of mice treated with single MAB IgG or combinations of 2 or more MAbs was confirmed by the isolation of virus from their brains. However, a few of the survived mice treated with 3 or 4 MAbs showed early signs of sickness, such as a slight dullness, consuming less food, and having slightly rough fur. These symptoms gradually disappeared and no virus was isolated from the brains of recovered mice collected 21 days after the virus inoculation. Figures 1 and 2 show the survivors (%) on different days p.i. with JEV strains 641686 and 691004 among the mice administered with single MAbs or with their combinations.

Discussion and Conclusions

The gpE is a major flaviviral antigen which binds to cellular receptors and mediates cell membrane fusion. It contains an array of epitopes that elicit virus neutralizing and non-neutralizing antibodies. The protective efficacy of an anti-gpE specific MAb is, therefore, directly related to its ability to neutralize the virus infectivity. The characterization of MAbs directed against gpE of JEV has earlier indicated a critical neutralization site that was recognized by all the 4 Hs MAbs on gpE of JEV strain 733913 and these MAbs protected the mice against lethal virus challenge. However, JEV group II strains were neither neutralized nor the mice infected with JEV group II strains protected by the Hs MAbs except for some increase in mice survival with MAB Hs-3. As these JEV group II strains could still be neutralized with some of the MAbs belonging to the NHs and Hx groups, although no protection was noticed with the MAbs given alone except for an increase in mice survival. Therefore, their MAbs combinations were examined for additive or enhanced protective effect.

In the present study, 70-75% protection was achieved with a combination of 4 MAbs i.e.: MAbs NHs-1, Hx-1, Hx-3 and Hs-3. Protection was only 20-40% with the 3 MAbs and none with single or 2 MAbs except for an increase in mice survival against 2 JEV group II strains 641686 and 691004 infections. Although no statistical comparisons of AST values obtained in control and experimental group, the increase in animal survival seemed to be directly related to the combinations of MAbs used. Addition of another MAB or 2 may produce a desirable effect (up to 100% protection) but this, however, remains to be clarified. Furthermore, JEV group II strains seemed to have emerged in different ecological situations over a number of years in the flavivirus endemic areas. A laboratory set-up for the selection of a neutralization-escape variant of the JEV strain 733913 against MAB Hs-1 showed that antibody pressure seemed to be one of the important factors in the evolution of
JEV group II strains. Therefore, the presence of epitopes unique to these strains cannot be ruled out and it will, therefore, be worthwhile examining this possibility by carrying out the protective experiments using MAbs prepared against some of the JEV group II strains. Interestingly, MAb Hx-3, which is non-neutralizing, also showed an increase in mice survival and/or protection when administered in combination with neutralizing MAbs. Studies by Gould et al. using 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 depends on the virus neurovirulence. Also, protection afforded by a non-neutralizing MAb against YFV was the indication of the involvement of other factors rather than neutralization alone which might be responsible for such an effect of MAb Hx-3 against JEV group II strains in mice. It was shown that MAbs can induce conformation-dependent changes in the viral epitopes. In the present study, therefore, the role of such changes in protection cannot be ruled out. In view of the fact that no specific therapies are available for the treatment of JE, the present findings gain importance in offering further possibilities of using antibodies in the treatment of JE cases. The results also indicate that NHs and Hx epitopes should be added to 3 Hs epitopes in a JEV vaccine that would, therefore, have an added advantage, particularly in endemic areas with JEV strain variations.

References

1. Westaway EG, Brinton MA, Gaidamovich S, et al. Flaviviridae. Intervirology 1985;24:183-92.
2. Vaughn DW, Hoke CH. The epidemiology of Japanese encephalitis: prospects for prevention. Epidemiol Rev 1992;14:197-221.
3. Monath TP, Heinz FX. Flaviviruses. In: Fields BN, Koepe DM, Howley PM, eds. Field’s fundamental virology. 3rd Ed. Philadelphia: Lippincott- Raven; 1996. pp. 961-1034.
4. Solomon T, Ni H, Beasley DW, et al. Origin and evolution of Japanese encephalitis virus in Southeast Asia. J Virol 2003;77:3091-8.
5. Rodrigues FM. Epidemiology of Japanese encephalitis in India: a brief overview. In: Proceedings of the National Conference on Japanese Encephalitis, New Delhi: Indian Council of Medical Research; 1984. pp. 1-9.
6. Hanna JN, Ritchie SA, Philips DA, et al. An outbreak of Japanese encephalitis in the Torres Strait, Australia, 1995. Med J Aust 1996;165:256-60.
7. Hanna JN, Ritchie SA, Philips DA, et al. Japanese encephalitis in North Queensland, Australia, 1998. Med J Aust 1999;170:533-66.
8. George S, Gourie-Devi M, Rao JA, et al. Isolation of West Nile virus from the brains of children who died of encephalitis. Bull WHO 1984;62:879-82.
9. Carey DE, Myers RM. Japanese encephalitis studies in Vellore, South India. Part III. Neutralizing activity of human serum. Indian J Med Res 1968;56:1330-9.
10. Peiris JSM, Porterfield JS, Roehring JT. Monoclonal antibodies against the flavivirus West Nile virus. J Gen Virol 1982;58:283-9.
11. Kimura-Kuroda J, Yasui K. Topographical analysis of antigenic determinants on envelope glycoprotein V3 (E) of Japanese encephalitis virus using monoclonal antibodies. J Virol 1983;45:124-32.
12. Gould EA, Buckley A, Barrett ADT, Cammaack N. 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 1986;67:591-5.
13. Kedarnath N, Dayaraj C, Sathe PS, et al. Monoclonal antibodies against Japanese encephalitis virus. Indian J Med Res 1986;84:125-33.
14. Cecilia D, Gadkari D, Kedarnath N, Ghosh SN. Epitope mapping of Japanese encephalitis virus envelope protein using monoclonal antibodies. J Virol 1988;62:2741-7.
15. Ghosh SN, Sathe PS, Sarthi SA, et al. Epitope analysis of Japanese encephalitis virus by monoclonal antibodies. Indian J Med Res 1989;89:368-75.
16. Gupta AK, Lad VJ, Koshy AA. Protection of mice against experimental Japanese encephalitis virus infection by neutralizing anti-glycoprotein E monoclonal antibodies. Acta Virol 2003;47:141-5.
17. Gupta AK, Koshy AA, Lad VJ. Enhanced protection of mice against Japanese encephalitis virus infection by combinations of monoclonal antibodies to glycoprotein E. Acta Virol 2011;55:165-8.
18. Gupta AK, Lad VJ, Koshy AA, Gadkari DA. Loss of virus specific epitopes on JE virus glycoprotein by acetone treatment. Indian J Med Res 2000;112:113-20.
19. Gupta AK, Lad VJ, Koshy AA. Protection against infection of group II Japanese encephalitis virus strains in mice. Indian J Virol 2006;17:9-14.
20. Gupta AK, Lad VJ, Koshy AA. 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. Acta Virol 2008;52:219-24.
21. Gupta AK, Lad VJ, Koshy AA. Early death of Japanese encephalitis virus-infected mice administered immunized a neutralizing cross-reactive monoclonal antibody against glycoprotein E. Acta Virol 2009;53:191-5.
22. Heinz FX, Berger R, Tuma W, Kunz C. A topological and functional model of epitopes on the structural glycoprotein of Tick-borne encephalitis virus defined by monoclonal antibodies. Virology 1983;126:525-37.
23. Mathews JH, Roehring JT. 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 1984;132:1533-7.
24. Kimura-Kuroda J, Yasui K. Protection of mice against Japanese encephalitis virus by passive administration with monoclonal antibodies. J Immunol 1988;141:3606-10.
25. Gore MM, Gupta AK, Ayachit VM, et al. Selection of a neutralization-escape variant of Japanese encephalitis virus using monoclonal antibody. Indian J Med Res 1990;91:231-3.
26. Cepica A, Yason C, Ralling G. The use of ELISA for detection of antibody induced conformational change in viral protein and its intermolecular spread. J Virol Meth 1990;28:1-14.
27. Gupta AK, Bhattacharyya S, Lad VJ, et al. Monoclonal antibody to Japanese encephalitis virus cross-reacting with histones present in the cell nuclei. Acta Virol 1992;36:401-11.
28. Gupta AK, Lad VJ, Sarthi SA, et al. An IgM monoclonal antibody to JE virus recognizing a cross-reacting epitope on nuclear histones. Indian J Med Res 1999;110:149-54.