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Monoclonal Antibodies against Japanese Encephalitis Virus Envelope Glycoprotein V3 (E)

Ashok Kumar SRIVASTAVA, Sachiko MATSUO and Akira IGARASHI

Department of Virology, Institute of Tropical Medicine, Nagasaki University, 12-4 Sakamoto-machi, Nagasaki 852, Japan

Yoshinori SOHDA and Hiroshi SHIKU

Department of Oncology, Nagasaki University School of Medicine, 12-4 Sakamoto-machi, Nagasaki 852, Japan

Abstract: In order to analyze epitopes of Japanese encephalitis (JE) virus envelope glycoprotein V3 (E) in relation to its molecular structure, 42 monoclones were obtained. They were characterized by immunoglobulin isotyping, neutralization (N) test, Western blotting after polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) under reducing and nonreducing conditions. Enzyme-linked immunosorbent assay (ELISA) was used to examine cross-reactivity of the monoclones to other strains of JE and flaviviruses, and 17 of them were classified into 5 groups. Strain-specific monoclones in group 1 contained 3 IgM, 2 IgG2a, and 1 IgG2b isotypes. Three IgM monoclones were (1-a-1) N-positive and linear, (1-a-2) N-positive and conformational, or (1-a-3) N-negative and conformational. Two IgG2a monoclones were N-negative and (1-b-1) linear, or (1-b-2) conformational, while 1 IgG2b monoclone (1-c) was N-positive and conformational. One monoclone in group 2 was JE virus-specific, IgM, N-positive and linear. Two N-negative monoclones in group 3 showed subgroup-specific reactivity to JE, Murray Valley encephalitis (MVE), West Nile (WN), and St. Louis encephalitis (SLE) viruses. One of them (3-a) was linear IgG1, and another (3-b) was conformational IgG2b. Group 4 monoclones cross-reacted to the subgroup and dengue type 2 (D2) viruses, and contained 2 IgM, 1 IgG2a, and 2 IgG2b isotypes. Two IgM monoclones were (4-a-1) N-positive and linear, or (4-a-2) N-negative and conformational. One IgG2a monoclone (4-b) was N-positive and linear, while 2 IgG2b monoclones were N-negative and (4-c-1) linear, or (4-c-2) conformational. Three N-negative monoclones in group 5 cross-reacted to all flaviviruses including D2 and yellow fever (YF) viruses, and were (5-a) highly conformational IgG1, (5-b) conformational IgG2a, and (5-c) conformational IgG2b. Remaining 25 monoclones showed various degrees of cross-reactivity to different flaviviruses.

Key words: Monoclonal antibodies, Japanese encephalitis virus, Cross-reactivity, Flaviviruses

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INTRODUCTION

Japanese encephalitis (JE) virus in a member of the family Flaviviridae with a single-stranded and positive sense RNA genome of 42 S (Westaway *et al.*, 1985), and formerly known as a mosquito-borne group B arbovirus (Friedman, 1968). Several flaviviruses, for example, JE, YF, dengue, and tick-borne encephalitis (TBE) viruses, have been counted among human pathogens (Shope, 1980; Monath, 1986). Multiple flavivirus infections have been prevalent in some part of Asia, and JE has been a serious public health problem because of its high mortality and grave sequelae (Miles, 1960; Umenai *et al.*, 1985). Dengue haemorrhagic fever (DHF) has been another serious public health problen in Southeast Asia because of large numbers of patients sometimes accompanied by shock and death (Halstead, 1966, 1980). The pathogenesis of DHF has been explained by hypersensitivity (Russell, 1971) or immune enhancement (Halstead, 1980) which could take place in the secondary infection by different serotypes of dengue viruses from the first infection. These theories have complicated the objectives of dengue vaccine development, JE vaccination, and serodiagnosis in the areas where multiple flavivirus infections have been prevalent.

Among flaviviruses, JE, WN, MVE, and SLE viruses constitute a subgroup by their cross-reactions in the hemagglutination-inhibition (HI) test (Clarke and Casals, 1958) and in the N test (Madrid and Porterfield, 1974; Porterfield, 1980; Chamberlain, 1980), while YF, dengue, and TBE viruses constitute each separate subgroup. Three structural proteins of flaviviruses, V1(M), V2(C) and V3(E), were first reported for dengue virus (Stollar, 1969), and later for other flaviviruses including JE virus (Shapiro *et al.*, 1971; Kitano *et al.*, 1974; Takegami *et al.*, 1982). The V3(E) of SLE virus was shown to contain 3 antigenic determinants; flavivirus cross-reactive, complex or subgroup specific, and serotype specific (Trent *et al.*, 1976; Trent, 1977). Monoclonal antibody technique (Kohler and Milstein, 1975) has been used to analyze antigenic structures of dengue viruses (Dittmar *et al.*, 1980; Henchal *et al.*, 1982), and antibody-dependent enhancement of WN virus replication (Peiris *et al.*, 1981). Heinz (1986) reviewed V3(E) epitope mapping of TBE and other flaviviruses. Monoclonal antibodies to JE virus have been developed to analyze V3(E) epitopes and classify several JE virus strains (Lin, 1982; Kimura-Kuroda and Yasui, 1983, 1986a, 1986b; Kobayashi *et al.*, 1984; Kobayashi *et al.*, 1986).

As the first step to analyze biological activities of JE virus V3(E) in relation to its nucleotide and deduced amino acid sequence (Sumiyoshi *et al.*, 1986; 1987), we developed monoclonal antibodies with their characterizations.

MATERIALS AND METHODS

Cells: Aedes albopictus clone C6/36 cells (Igarashi, 1978) were used to propagate JE and other flaviviruses. The cells were grown as mass culture in suspension using 1 liter volume of spinner bottles at 28℃ with cell growth medium of Eagle's minimal essential

medium supplemented by 0.2 mM each of nonossential amino acids (Eagle, 1959) and 10% heat-inactivated fetal calf serum (FCS). Between 6-12 hours before virus inoculation, microcarrier (Cytodex 1, Pharmacia, Sweden) was added to the culture at final concentration of 2 mg/ml to attach the cells on it. BHK-21 cells were grown at 37℃ with the same growth medium as above, and used in virus infectivity assay and N test. A mouse myeloma cell line, P3-NS 1/1 Ag4-1 (NS-1), was used for monoclonal antibody preparation. The cells were grown in suspension at 37℃ in humidified 5% CO_2 atmosphere with RPMI 1640 medium (Nissui Seiyaku, Japan) supplemented with 10% heat-inactivated FCS (GIBCO, USA) and 20μg/ml 8-azaguanine as described by Kato *et al.* (1985).

Viruses: Origin of JE virus strains, Nakayama-Yoken, JaGAr-01, and JaOArS982, was described by Hori (1986). Seeds of MVE, WN (Eg101 strain), SLE, D2 (New Guinea B strain), and YF (17D strain) viruses had been kept in the Department of Virology, Institute of Tropical Medicine, Nagasaki University. Each virus was concentrated and purified from infected C6/36 cell culture fluid by polyethylene glycol (PEG) precipitation and ultracentrifugation as described before (Srivastava *et al.*, 1987).

Immunization of mice: BALB/c × C57BL/6 (CB6F1) mice were immunized by 5 intraperitoneal injections with 1 week intervals, using purified JE virus (0.75mg/ml, 0.1ml/mouse/dose) mixed with an equal volume of Freund's complete adjuvant for the first injection or Freund's incomplete adjuvant for the subsequent injections. In AP series, the mice were boosted with purified virus (0.786mg/ml, 1ml/mice), 1 week after the last immunization. While in AI series, the interval between the last immunization and booster was 2 months, and the mice were boosted with the same amount of purified virus disrupted by Triton X-100 (TX-100) with virion protein to TX-100 ratio of 1:10 at 20℃ for 10 minutes (Srivastava and Igarashi, 1987).

Cell fusion: The procedure developed by Köhler and Milstein (1975) was followed. Three days after the booster, immunized mouse was sacrificed by bleeding under anesthesia, serum was separated from the blood and stored at −20℃, and spleen was removed to prepare single cell suspenstion under sterile condition. Immune spleen cells (10^8) and NS-1 cells (10^6) were mixed, washed twice in RPMI-1640 medium by low-speed centrifugation, and treated with 200μl of 49% PEG (Mw 4000, Katayama Chemicals, Japan) at 37℃ for 2 minutes. The cells were washed as above and suspended at a concentration of 5×10^5 cells/ml for selection of hybridoma cells in HAT medium (RPMI-1640 medium supplemented with 10 mM hypoxanthine, 4 mM aminopterin, 1.6 mM thymidine, and 20% FCS). The cell suspension was seeded in 100μl aliquots into flat-bottom 96 well microplates (Falcon, USA) and incubated at 37℃ in humidified 5% CO_2 atmosphere. Three days latter, the plates were observed under a microscope to select the wells containing a single surviving cell or a single colony which apparently came out of a single hybrid cell, and 100μl of HAT medium was added to each well. Seven days later, 100μl of the medium in each well was replaced by 100μl of HT medium (HAT medium from which aminopterine was removed). Ten days after, the culture fluid from the wells was screened for anti-JE antibodies by the ELISA. ELISA positive clones were transfered to 24-well

plates (Noucatom, Denmark) and further expanded in 25 cm^2 flasks (Corning, USA). When monoclonality of hybridoma cell lines was dubious, limiting dilution was carried out to obtain monoclones. All the established antibody-positive hybridoma cell lines were stored in a liquid nitrogen tank.

ELISA: Indirect micro ELISA (Voller *et al.*, 1976) was used to screen and assay anti-JE and other flavivirus ELISA antibodies. The 96-well microplates were coated with purified virion (50-80 μg/ml in coating buffer, 100μl/well) at 4℃ overnight. The wells were emptied and washed with PBS-T (phosphate buffered saline containing 0.05% Tween 20 and 0.01% NaN_3) 3 times 3 minutes each. The wells were reacted at 37℃ for 1 hour with 100μl/well of hybridoma culture fluid (undiluted, and diluted to 1:10, and 1:100), or immune mouse sera (diluted to 1:100, 1:1000, and 1:10000), along with a serially diluted standard anti-JE polyclonal mouse serum. PBS-T was used to dilute above specimens and horseradish peroxidase (HRPO)-conjugated goat IgG in the next step. The wells were emptied, washed as above, and reacted at 37℃ for 1 hour with 100μl/well of HRPO-conjugated anti-mouse immunoglobulin goat IgG (Cappel, USA). For total immunoglobulin assay, heavy and light chain reactive HRPO-conjugate was used at 1:1000 dilution, while μ-chain specific HRPO-conjugate at 1:200 dilution was used for IgM antibody assay. The wells were emptied, washed as above, and HRPO reaction was performed at room temperature for 1 hour in the dark with substrate solution of 0.5 mg/ml of *o*-phenylene diamine and 0.02% of H_2O_2 in 0.05 M citrate-phosphate buffer, pH 5.0. The reaction was stopped by adding 75 μl/well of 4 N H_2SO_4, and optical desnity (OD) at 490 nm was recorded by micro ELISA autoreader with reference wavelength at 630 nm. Titers of test specimens were calculated by comparing their OD with those by serially diluted standard positive serum as described before (Igarashi *et al.*, 1981; Morita *et al.*, 1982). Standard anti-JE polyclonal mouse serum was prepared by repeated intraperitoneal inoculation of purified JE virus grown in suckling mouse brains.

SDS-PAGE: Slab gel method (Studier, 1973) with discontinuous buffer system (Laemmli, 1970) was used with 15% gel (acrylamide to bisacrylamide ratio of 30:0.8) in 1.5 mm thickness. Purified JE virion was solubilized under reducing condition using 5% 2-mercaptoethanol (2ME), or nonreducing condition using 0.1 M iodoacetamide (IAA), in 0.125 M Tris-HCl and 1% SDS, pH 6.8, at 100℃ for 1 minute before electrophoresis (Durbin and Stollar, 1984).

Western blotting: Structural proteins of JE virus separated by SDS-PAGE were electrophoretically transfered to nitrocellulose membrane (Towbin *et al.*, 1979; Burnette, 1981; Naser and Miltenburger, 1983) with some modifications (Srivastava *et al.*, 1987). The membrane was inactivated with 3% casein and 0.01% NaN_3 in PBS (phosphate buffered saline) at room temperature for 45 minutes, washed in PBS and cut into small strips. Each strip was reacted at 37℃ for 3 hours with undiluted monoclonal culture fluid or standard anti-JE polyclonal mouse serum diluted to 1:1000 in PBS. The membrane strips were washed in PBS and reacted at 37℃ for 2 hours with HRPO-conjugated anti-mouse IgG (heavy and light chain reactive) goat IgG (Cappel, USA) diluted to 1:1000 in PBS. the membrane

strips were washed with PBS, and reactiviry of anti-JE antibodies to JE virus structural proteins was visualized by HRPO reaction with 0.03% 4-chloro-1-naphthol and 0.03% H_2O_2 in PBS at room temperature for appropriate time until definite color was developed.

N test of JE virus: The procedure duscribed by Hashimoto *et al.* (1971) was somewhat modified, using diluent of 5% FCS in Eagle's medium. JE virus, JaOArS982 strain-infected C6/36 cell culture fluid, at 3 different dilutions was mixed with an equal volume of monoclonal culture fluid (undiluted, and diluted to 1:4, 1:10, 1:40 and 1:100), immune mouse sera and the standard anti-JE mouse serum as positive control (diluted to 1:100, 1:1000, and 1:10000), and virus diluent as negative control. The mixtures were incubated at 37℃ for 1 hour, and inoculated to BHK21 cells grown on 24 well plates (0.1 ml/well), after removing cell growth medium. Adsorption was carried out at 37℃ for 2 hours and the cells were covered by 1 ml/well of 1.5% methylcellulose in maintenance medium (1% FCS in Eagle's medium). After 5 days of incubation at 37℃ in humidified 5% CO_2 atmosphere, the overlay medium was removed by washing with PBS. The cells were fixed with cold methanol at −20℃ for 30 minutes and stained with 0.1% Trypan blue to reveal the plaques. The N test was scored as positive when more than 50% of plaque-reduction was observed compared with the negative control. Reciprocal of the highest dilution of test specimen with N-positive result was taken as the N titer.

Immunoglobulin isotyping: The gel-diffusion method (Ouchterlony, 1958) and mouse sub-isotyping kit (Bio-Rad Laboratories, USA) were used.

Reagents: Acrylamide, bis-acrylamide, TX-100, 2ME and IAA were the products of Wako Pure Chemicals.

RESULTS

Formation of monoclonal hybridoma cell lines secreting anti-JE V3(E) antibodies

Altogether 5 cell fusions were performed, and only 2 fusions (AP and AI series) were successful to establish monoclonal hybridoma cell lines secreting anti-JE V3(E) antibodies in their culture fluid. In remaining 3 fusions, antibody-secreting cells were not produced or those cells were lost during the passages. After recloning, the number of monoclonal hybridoma cell lines secreting anti-JE antibodies was 22 from AP and 20 from AI series, respectively. Results of their characterization by immunoglobulin isotyping and N test were summarized in Table 1. A monoclone was considered as N-positive when its ratio of N to ELISA titer was equal to or greater than 1.0. On the other hand, monoclenes were considered as N-negative when their ratio of N to ELISA titer was under 1.0. By the isotyping, there were 2 IgG1 (AI series), 13 IgG2a (1 AP, and 12 AI series), 11 IgG2b (6 AP, and 5 AI series), 1 IgG3 (AP series), 14 IgM (13 AP, and 1 AI series), and 1 IgA (AP series) monoclones. The result indicated preponderance of IgM isotypes in AP series, in contrast to IgG2a in AI series, and was consistent with the ELISA on immune mouse serum in each series (Table 2). Anti-JE IgM titer in AP series was 81% of anti-JE immunoglobulin titer and was 7.8 times higher than in AI series, in

which anti-JE IgM titer was 26.9% of anti-JE immunoglobulin titer. Anti-JE immunoglibulin titer was 2.6 times higher in AP than in AI series, but the difference was less than IgM titer. Except one N-positive IgG2a monoclone (5%), all the other 19 monoclenes (95%) from AI series were N-negative. On the other hand, 17 monoclones (77.3%) from AP series were N-positive, including 11 IgM, 3 IgG2b, and each 1 of the IgG2a, IgG3, and IgA isotypes. This finding indicaties that more clones producing N antibodies were stimulated in AP than in AI series. However, immune mouse sera from both series showed similar N titers with or without 2ME treatment, indicating that most of the N antibodies in these sera were IgG class produced during immunization before the booster (data not shown).

Table 3 shows the classification of monoclones according to their epitope reactivity in the Western blotting and isotypes. The epitopes of 2 (4.8%) monoclones in AI series (IgG1 and IgG2a) were considered as highly conformational, because they did not react with V3(E) in the Western blotting even under nonreducing condition, although they

Table 1. Classification of monoclones obtained in AP and AI series by N-test and immunoglobulin isotyping

Immuno-globulin isotype	N-test* on monoclones in cell fusion						All total
	AP			AI			
	(+)	(−)	Total	(+)	(−)	Total	
IgG1	0	0	0	0	2	2	2
IgG2a	1	0	1	1	11	12	13
IgG2b	3	3	6	0	5	5	11
IgG3	1	0	1	0	0	0	1
IgM	11	2	13	0	1	1	14
IgA	1	0	1	0	0	0	1
Total	17	5	22	1	19	20	42
(% to total)	(77.3)	(22.7)		(5)	(95)		

Figures represent number of monclones.
* Positive (+) when N to ELISA titer ratio was over 1.0; negative (−) when the ratio was under 1.0.

Table 2. Anti-JE ELISA titers in mouse serum collected before cell fusion

Fusion series	Immunoglobulin ELISA titer	
	Total	IgM (% to total)
AP	15,400	12,480 (81.0)
AI	5,950	1,600 (26.9)
AP/AI	2.59	7.8

Table 3. Classification of monoclones in AP and AI series by their reactivity in Western blotting and isotypes

Immuno-globulin isotype	Epitope reactivity* of monoclones in cell fusion							All total
	AP			AI				
	Lin	Conf	Total	Lin	Conf	Hi-Conf	Total	
IgG1	0	0	0	1	0	1	2	2
IgG2a	0	1	1	7	4	1	12	13
IgG2b	1	5	6	0	5	0	5	11
IgG3	0	1	1	0	0	0	0	1
IgM	5	8	13	0	1	0	1	14
IgA	0	1	1	0	0	0	0	1
Total (% to total)	6 (27.3)	16 (72.7)	22	8 (40)	10 (50)	2 (10)	20	42

Figures represent number of monoclones.

* Linear (Lin) when reactive both under reducing and noreducing conditions. Conformational (Conf) when reactive only under noreducing condition. Highly conformational (Hi-conf) when no reaction under both reducing and nonreducing condition.

showed moderete to high ELISA titers. While, the epitopes of 26 (61.9%) monoclones (16 AP, and 10 AI series) were considered as conformational, because they reacted with V3 (E) in the Western blotting only under nonreducing condition. Among these conformational monoclones, there were 8 IgM, 5 IgG2b, and each 1 of IgG2a, IgG3 and IgA isotypes in AP series, in contrast to 5 IgG2b, 4 IgG2a, and 1 IgM isotypes in AI series. The epitopes of remaining 14 (33.3%) monoclones (6 AP, and 8 AI series) were considered as linear, because they reacted with V3 (E) by the Western blotting even under reducing condition. Among these linear monoclones, there were 5 IgM and 1 IgG2b isotypes in AP series, in contrast to 7 IgG2a and 1 IgG1 isotypes in AI series. More than half (58.3%) of the IgG2a monoclones from AI series and one-third (38.5%) of the IgM monoclones from AP series were linear. In contrast, 83.3% (AP series) and all (AI series) of IgG2b monoclones were conformational.

Cross-reactivity and grouping of monoclonal antibodies

A monoclone was considered as cross-reactive to a heterologous virus, when its ELISA titer to the virus was equal to or greater than 50% of its ELISA titer to the homologous virus, JaOArS982 strain of JE virus, which was used to immunize mice. By cross-reactivity in the ELISA, 17 out of the 42 monoclones were classified into 5 groups as shown in Table 4. Group 1 contained 6 strain-specific monoclones with 3 IgM, 2 IgG2a, and 1 IgG2b isotypes. By the N test and Western blotting, 3 IgM monoclones were (1-a-1) N-positive and linear [clone AP-98], (1-a-2) N-positive and conformational [clone AP-97], and (1-a-3) N-negative and conformational [clone AP-244]. Two IgG2a monoclones were N-negative, and (1-b-1) linear [clone AI-110-2], or (1-b-2) confromational [clone AI-

101]. One IgG2b monoclone (1-c) was N-positive and conformational [clone AP-124]. One monoclone in group 2 was JE-specific, IgM, N-positive, and linear [clone AP-104]. Two monoclones in group 3 were subgroup-specific to JE, MVE, WN and SLE viruses, N-negative, and (3-a) linear IgG1 [clone AI-901], or (3-b) conformational IgG2b [clone AI-112]. Group 4 monoclones cross-reacted to subgroup and D2 viruses, and contained 2 IgM, 1 IgG2a, and 2 IgG2b isotypes. Two IgM monoclones were (4-a-1) N-positive and linear [clone AI-119], or (4-a-2) N-negative and conformational [clone AI-315], while one IgG2a monoclone (4-b) was N-positive and linear [clone AI-300]. Two IgG2b monoclones were N-negative, and (4-c-1) linear [clone AP-121], or (4-c-2) conformational [clone AI-402]. Group 5 monoclones cross-reacted to all the flaviviruses including D2 and YF viruses, were N-negative, and contained each 1 of the (5-a) highly conformational IgG1 [clone AI-904], (5-b) conformational IgG2a [clone AP-804], and (5-c) conformational IgG2b [clone AI-504]. Remaining 25 monoclones showed various patterns of cross-reactivity and characterizations, so that they were not listed up in Table 4.

Table 4. Grouping of representative monoclones by their biological properties

Group	Cross-reaction in ELISA*	Immuno-globulin isotype	N test to JE virus**	Reactivity of epitope***	Mono-clone code
1−a−1	Strain	IgM	+	Lin	AP−98
1−a−2	Strain	IgM	+	Conf	AP−97
1−a−3	Strain	IgM	−	Conf	AP−244
1−b−1	Strain	IgG2a	−	Lin	AI−110−2
1−b−2	Strain	IgG2a	−	Conf	AI−101
1−c	Strain	IgG2b	+	Conf	AP−124
2	JE	IgM	+	Lin	AP−104
3−a	Subgroup	IgG1	−	Lin	AI−901
3−b	Subgroup	IgG2b	−	Conf	AI−112
4−a−1	Subgroup+D2	IgM	+	Lin	AI−119
4−a−2	Subgroup+D2	IgM	−	Conf	AI−315
4−b	Subgroup+D2	IgG2a	+	Lin	AI−300
4−c−1	Subgroup+D2	IgG2b	−	Lin	AP−121
4−c−2	Subgroup+D2	IgG2b	−	Conf	AI−402
5−a	Flavivirus	IgG1	−	Hi-Conf	AI−904
5−b	Flavivirus	IgG2a	−	Conf	AI−804
5−c	Flavivirus	IgG2b	−	Conf	AI−504

*Cross-reactive when ELISA titer to other virus or strain was over 50% to JaOArS982 strain
**Positive (+) for N/ELISA ratio>1.0, otherwise negative (−)
***Linear (Lin): reactive both under reducing and nonreducing, conformational (Conf): reactive only under nonreducing, highly conformational (Hi-Conf): no reaction under both reducing and nonreducing conditions.

DISCUSSIONS

Kimura-Kuroda and Yasui (1983, 1986a, b) isolated IgG2b class of monoclonal antibodies against JE virus V3(E) and classified them into 8-9 groups by competitive binding assay and cross-reactivity in the ELISA, N test and HI test, showing at least 8-9 independent epitopes on JE virus V3(E). Kobayashi *et al.* (1984) classified several JE virus strains into 6 groups by their reactivity in the HI test. Using strain-specific monoclenes, Yasui's group classified JE virus strains into 3 subtypes of Nakayama, JaGAr-01, and others (Kobayashi *et al.*, 1986). However, they did not mention reactivity of monoclones in the Western blotting. Our previous study showed that the reactivity to JE virus V3(E) in the Western blotting was significantly reduced for anti-JE polyclonal serum or lost for most of the monoclones developed by Kobayashi *et al.* (1984), when the virus was disrupted under reducing condition before SDS-PAGE (Srivastava *et al.*, 1987). The results indicated that the epitopes of JE virus V3(E) recognized by conventional immunization were mostly conformational.

In the present study, we isolated 42 monoclenes from 2 fusions with different booster immunizations. Booster with whole virion 1 week after the last immunization (AP series) produced primarily IgM monoclones, most of which were N-positive. On the other hand, booster with TX-100 disrupted virion 2 months after the last immunization (AI series) produced primarily IgG2a monoclones, most of which were N-negative. Whether this difference is due to the different booster immunogen or interval between the last immunization and the booster may be interesting to investigate further. The ratio of linear in total monoclones was similar for both AP and AI series, and most (AP series) and all (AI series) of IgG2b monoclones were conformational. So that, the chance to obtain conformational IgG2b monoclone is high, regardless booster immunogen and interval between the last immunization and booster. While, more than one-third of IgM monoclones in AP series and more than half of IgG2a monoclones in AI series were linear.

Cross-reactivity test by the ELISA indicated at least 6 different strain-specific epitopes on JE virus V3 (E), linear or conformational, N-positive or N-negative. We could not isolate any N-positive monoclones which were subgroup-sepcific or flavivirus cross-reacting. However, IgM and IgG2a monoclones, which cross-reacted to the subgroup and D2 virus, were N-positive and linear. The epitopes of these monoclones may be good candidates of the second generation subunit vaccine, if they can neutralize these viruses, as well as JE virus, and protect against these viruses.

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日本脳炎ウイルス外被膜糖タンパク V3(E) に対する単クローン抗体

Ashok Kumar SRIVASTAVA, 松尾幸子, 五十嵐 章（長崎大学熱帯医学研究所ウイルス学部門）
珠玖 洋, 早田義典（長崎大学医学部腫瘍医学教室）

日本脳炎（JE）ウイルス外被膜糖タンパク V3(E) のエピトープと分子構造の関係を解析するために単クローン抗体42個を分離し，それぞれの性状を免疫グロブリンのアイソタイプ，JE ウイルスに対する中和活性，還元並びに非還元状態で破壊してドデシル硫酸ソーダ存在下のポリアクリルアミドゲル電気泳動で分離したウイルスタンパクに対するウエスタンブロッチング法での反応性で検討した．JE ウイルス株並びフラビウイルスに対する単クローン抗体の反応性を免疫酵素測定法で測定し，17個の単クローン抗体を5群に分類した．株特異的反応性を示す第一群には，3個の IgM，2個の IgG2a，と1個の IgG2b 抗体が含まれ，IgM 抗体は(1-a-1)中和陽性で線状エピトープに対するもの，(1-a-2)中和陽性で立体的エピトープに対するもの，(1-a-3)中和陰性で立体的エピトープに対するものに分類され，IgG2a 抗体は中和陰性で(1-b-1)線状エピトープに対するものと(1-b-2)立体的エピトープに対するものに分類され，IgG2b 抗体(1-c)は中和陽性で立体的エピトープと反応した．JE ウイルスに特異的な第二群には中和陽性で線状エピトープに対する IgM 抗体が存在した．第三群に含まれる2個の抗体は中和陰性で JE，マレー渓谷脳炎，西ナイル，セントルイス脳炎ウイルスから成るフラビウイルス中の亜群に対して反応し，(3-a)線状エピトープに対する IgG1 抗体と (3-b)立体的エピトープに対する IgG2b 抗体であった．第四群には亜群と2型デングウイルスに対して反応する2個の IgM，1個の IgG2a と，2個の IgG2b 抗体が含まれ，IgM 抗体は(4-a-1)中和陽性で線状エピトープに対するものと(4-a-2)中和陰性で立体的エピトープに対するものに分類され，IgG2a 抗体(4-b)は中和陽性で線状エピトープに反応し，IgG2b 抗体は中和陰性で(4-c-1)線状エピトープに対するものと(4-c-2)立体的エピトープに対するものであった．第五群に属する3個の中和陰性抗体は2型デングウイルスと黄熱ウイルスを含む全てのフラビウイルスに交差反応性を示し，(5-a)極めて立体的なエピトープに対する IgG1,(5-b)立体的エピトープに対する IgG2a と，(5-c)立体的なエピトープに対する IgG2b に分類された．

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