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BRIEF COMMUNICATION

ANTIGENIC ANALYSIS OF JAPANESE ENCEPHALITIS VIRUS ISOLATED IN HOKKAIDO WITH MONOCLONAL ANTIBODIES

Kenichi OCHIAI, Ikuo TAKASHIMA and Nobuo HASHIMOTO

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Japanese encephalitis (JE) virus is a mosquito-borne flavivirus and causes one of the most serious encephalitis in human and horse, and abortion in pigs. The virus is spread over a wide area of East Asian countries and JE is a major public health concern in Thailand, China and India[8]. It is still unknown how the virus survives the winter in the northern temperate zone while the vector mosquito is inactive[5]. Hokkaido, northern island of Japan is located within the ecotone of the virus and provides the most adverse climatic conditions for the survival of the JE virus in nature. Thus, Hokkaido has been considered as a suitable place to study the over-wintering mechanism of the virus[1,4]. In recent years, from 1984 to 1986, the outbreaks of JE abortion and inapparent infection of the virus struck the swine population in Hokkaido[7]. Three JE virus strains were isolated from aborted swine fetuses. Our previous seroepidemiological study suggests that the virus pass the winter in distinct endemic foci in Hokkaido[7]. Antigenic analysis of these strains is crucially important in order to know how the Hokkaido strains differ from the strains isolated in Honshu, main island of Japan. This study describes the antigenic comparison of JE viruses isolated in Hokkaido and Honshu with monoclonal antibodies and discusses the ecological implications of the results.

JE virus strains used in the study are shown in Table 1. These include 6 Honshu strains ; Nakayama, JaGAr-01, MI-351, 83090, 83091 and Akita, and 3 Hokkaido strains ; Imagane, Kamiiso and Saroma. West Nile virus Egypt 101 strain was used as another flavivirus. Monoclonal antibodies were prepared against the JE virus Imagane strain isolated from swine fetus in 1984[7]. Ten % brain homogenate was prepared in the phosphate buffered saline (PBS, pH ; 7.2) from a suckling mouse

Department of Veterinary Public Health, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

Table 1. Japanese encephalitis virus strains used in the study

Virus Strain	Isolation history		
	Year	Location	Origin
Japanese encephalitis			
Nakayama	1935	Tokyo	Human spinal fluid
JaGAr-01	1959	Gunma	*Culex tritaeniorhynchus*
MI-351	1979	Ibaraki	*Culex tritaeniorhynchus*
83090	1983	Yokohama	*Culex tritaeniorhynchus*
83091	1983	Yokohama	*Culex tritaeniorhynchus*
Akita	1983	Akita	Swine fetus
Imagane	1984	Hokkaido	Swine fetus
Kamiiso	1985	Hokkaido	Swine fetus
Saroma	1986	Hokkaido	Swine fetus
West Nile			
Egypt 101	1950	Egypt	Human blood

previously inoculated with the virus. The homogenate was centrifuged at 4,000xg for 20 min and the supernatant was diluted in PBS. The diluted virus suspension (3.8×10^3 PFU / 0.1ml) was inoculated directly into the spleen of BALB / c mice. Three days after inoculation, spleen cells from the mice were fused with mouse myeloma cells (SP 2/0 AG14) with the aid of polyethylene glycol 1,000 according to a previous paper[6]. Production of the antibody was screened by using indirect immunofluorescence antibody (IFA) test and C6/36 cells infected with Imagane strain as antigen[7]. Antibody producing cell lines were cloned three times in soft agar and monoclonal antibody in culture fluid was concentrated and used for the test. Reaction patterns of the antibodies were examined against each virus strain grown in C6/36 cells by the IFA test.

Monoclonal antibodies were also provided from Drs. J. KIMURA–KURODA and K. YASUI of Tokyo Metropolitan Institute for Neurosciences. These included 301 specific to flaviviruses, 503 specific to JE viruses, N–13 specific to JE virus strain Nakayama and 401 specific to JE virus strain JaGAr–01. These monoclonal antibodies were known to differentiate the JE virus strains into three types; Nakayama, JaGAr–01 and another types[2,3]. All of 3 Hokkaido strains and 6 Honshu strains reacted to flavivirus specific 301 and JE specific 503 (Table 2). Nakayama specific N–13 only reacted to Nakayama strain and JaGAr–01 specific 401 only to JaGAr–01 strain. The result shows that 3 Hokkaido strains and 4 Honshu strains (MI–351, 8390, 83091, and Akita) belong to another type distinct from Nakayama and JaGAr–01 types.

Twenty six clones of anti-Imagane monoclonal antibodies were established and the

Table 2 . Reaction of Japanese encephalitis virus strains isolated in Hokkaido with anti-JE virus monoclonal antibodies.

Monoclonal antibody (specificity)	IFA titer to JE virus[1]								
	Imagane	Kamiiso	Saroma	MI-351	83090	83091	Akita	Nakayama	JaGAr-01
301 (flavivirus specific)	160	160	80	80	80	80	80	80	160
503 (JE virus specific)	320	80	160	80	80	160	80	40	160
N-13 (Nakayama specific)	<10	<10	<10	<10	<10	<10	<10	100	<10
401 (JaGAr-01 specific)	<10	<10	<10	<10	<10	<10	<10	<10	800

1) JE virus was inoculated onto C6/36 cells, and cells were fixed with cold acetone and used for IFA test. IFA titer was expressed as the reciprocal of the highest dilution of the antibody-containing culture fluid showing a positive reaction.

Table 3. Reaction patterns of anti-Imagane monoclonal antibodies to various JE virus strains in IFA test.

Monoclonal antibody		IFA reaction[1] to									
Group	No.	Imagane	Kamiiso	Saroma	83091	JaGAr-01	Nakayama	MI-351	83090	Akita	West Nile
1	JE 1	+	+	+	+	+	+	+	+	+	−
	JE 2	+	+	+	+	+	+	+	+	+	−
	JE 3	+	+	+	+	+	+	+	+	+	−
	JE 4	+	+	+	+	+	+	+	+	+	−
	JE 5	+	+	+	+	+	+	+	+	+	−
	JE 6	+	+	+	+	+	+	+	+	+	−
2	JE 7	+	+	+	+	+	−	+	+	+	−
	JE 8	+	+	+	+	+	−	+	+	+	−
	JE 9	+	+	+	+	+	+	−	+	+	−
	JE10	+	+	+	+	+	+	+	−	+	−
	JE11	+	+	+	+	−	+	+	+	−	−
	JE12	+	+	+	+	+	+	+	−	−	−
	JE13	+	+	−	+	+	+	+	+	−	−
	JE14	+	+	+	+	+	+	−	+	−	−
	JE15	+	+	+	+	+	−	−	−	−	−
	JE16	+	+	−	+	+	+	−	−	−	−
	JE17	+	+	+	+	+	−	−	−	−	−
	JE18	+	+	+	+	+	−	−	−	−	−
	JE19	+	−	+	+	+	−	−	−	−	−
	JE20	+	+	+	−	+	−	−	−	−	−
	JE21	+	+	+	−	−	+	−	−	−	−
3	JE22	+	+	+	−	−	−	−	−	−	−
	JE23	+	+	−	−	−	−	−	−	−	−
	JE24	+	−	−	−	−	−	−	−	−	−
	JE25	+	−	−	−	−	−	−	−	−	−
4	JE26	+	+	+	+	+	+	+	−	−	+

1) IFA reaction was expressed as (+); titer ≥1:10 and (−); titer <1:10.

reaction patterns were tested against each virus strain in the IFA test to see the antigenic relation among JE virus strains (Table 3). Six monoclonal antibodies of group 1, JE 1 to 6, reacted to all 9 JE viruses. Fifteen of the monoclonal antibodies of group 2, JE 7 to 21, showed variable reaction patterns to each of JE virus. Monoclonal antibodies of group 3 showed specific reaction to Hokkaido strains. JE 22 showed a reaction to 3 Hokkaido strains, which suggests at least a common antigenic determinant existing among the Hokkaido strains. JE 23 reacted to Imagane and Kamiiso strains. JE 24 and 25 showed reaction only to homologous Imagane strain. JE 26 of group 4 reacted to most of JE virus strains and also to the West Nile virus.

The results of the antigenic analysis show that antigenicity of Hokkaido JE virus strains was different from that of the JE standard strains (Nakayama and JaGAr-01). The results also suggest that the Hokkaido strains had distinct antigenic determinants which were not detected on Honshu strains. The unique antigenic characteristics of Hokkaido strains may reinforce our previous assumption that indigenous JE viruses pass the winter in distinct endemic foci in Hokkaido[7].

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