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TWO-WAY CROSS-PROTECTION BETWEEN WEST NILE AND JAPANESE ENCEPHALITIS VIRUSES IN BONNET MACAQUES

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Summary. – Cross-protection between Japanese encephalitis (JE) and West Nile (WN) viruses was tested in bonnet macaques (*Macaca radiata)* immunized either with JE virus (JEV) or WN virus (WNV). JEV immunized monkeys were challenged by intranasal (i.n.) route with WNV and vice versa. Four control unimmunized monkeys were similarly infected either with WNV or JEV. Two of three control monkeys infected with WNV, developed paralysis followed by death. Virus was recovered from the central nervous system (CNS) of the both dead control monkeys and the histopathological examination of CNS revealed changes suggestive of viral encephalitis. The control monkey infected with JEV developed encephalitis and the virus was recovered from the blood and CNS. All the 3 JEV-immunized monkeys withstood WNV challenge, whereas only 2 of the 5 WNV immunized monkeys withstood the chalenge with JEV. Out of 3 WNV-immunized monkeys surviving challenge with JEV, 2 revealed symptoms suggestive of mild encephalitis followed by complete recovery. The third monkey died on the 60th day post-infection (p.i.) without any symptoms and virus was recovered only from the olfactory lobe. These studies indicate that the immunization with JEV protects the bonnet macaques against WNV, whereas the WNV immunization only reduces the severity of the disease due to JEV.

Key words: flaviviruses; cross-protection; bonnet macaques

Introduction

The mosquito-borne JE and WN flaviviruses are known to cause human infections in India (Carey and Mayers, 1968; Work, 1971). Both these viruses have been isolated from the same species of mosquitoes collected in the same region (Carey *et al.,* 1968). The simultaneous prevalence of these antigenically-related viruses in some parts of the country has been of epidemiological inte-

rest particularly due to the cross-immune response occuring in the human residents in the affected localities (Carey and Myers, 1968). The present studies were, therefore, undertaken to evaluate two-way cross-protection, if any, between JE and WN viruses in bonnet macaques (*Macaca radiata*) employing intranasal (i.n.) route for virus challenge.

Materials and Methods

Intranasally-adapted (Ghosh *et al.*, 1984) strains of JEV (strain No. 733913, isolated from the brain of a fatal human case in West India) and WNV (strain No. 68856, isolated from a wild-caught bat in Karnataka, India) were employed for virus challenge. The stocks of the virus were prepared from infected suckling mouse brains and were titrated in 2-3 days old infant mice group by intracerebral (i.c.) route. Bonnet monkeys 2-3 years old were employed for immunization against JEV (3 monkeys) and WNV (5 monkeys). Four doses (1.0 ml each) of formalin-killed JEV (733913) or WNV (68856) were administered at weekly intervals followed by four doses of live JEV (2.8 dex) and WNV (3 dex) respectively, by subcutaneous (s.c.) route. Four unimmunized monkeys served as controls.

At challenge, JEV or WNV (2.3 dex) was administered by i.n. instillation to both the immune and non-immune monkeys following the procedures described by Gopinath *et al.* (1978) and Ghosh *et al.* (1990). Monkeys were observed daily for the symptoms of encephalitis and the rectal temperatures were recorded. For the detection of viraemia in the monkeys, blood samples collected at staggered intervals from p.i. day 1 to 17 were inoculated into 2 day-old suckling mice by the i.c. route. The serum specimens collected were also tested for the presence of neutralizing (N) (Prasada Rao *et al.*, 1982) and haemagglutination-inhibition (HI) antibodies (Clarke and Casals, 1958). Organs (brain, spinal cord, olfactory lobe, lung, spleen and kidney) collected from dead or sacrificed monkeys, were processed for virus isolation in mice (George *et al.*, 1984) and the mouse brains of sick mice were tested in quick complement fixation test for confirmation of the virus identity (Pavri and Shaikh, 1966). Histopathological studies were carried out on the preserved organs (in 10 % formal saline) after cutting the paraffin-embedded sections and staining with haematoxylin and eosin.

Results

Monkeys challenged with WNV

Low-grade viraemia was detected in two (Nos. B20 and B32) of the three nonimmune monkeys on days 5 and 6 p.i. followed by symptoms suggestive of viral encephalitis on day 8. Both the monkeys succumbed to WNV infection on day 10. Histopathological examination of the CNS revealed changes suggestive of viral encephalitis (Ghosh *et al.*, 1990). WNV was isolated from the CNS *viz.* brain, olfactory lobe and spinal cord of both the monkeys. Interestingly, the third monkey (No. B26) which had low titres of HI and N antibodies against JEV prior to challenge, survived and did not show any overt illness. However, this monkey and other two control monkeys did not develop antibodies to WN and JE viruses up to days 25 and 7 p.i. respectively (Table 1).

Viraemia was found on day 4 p.i. in only one (No. B22) of three JEV immunized monkeys challenged with WNV. This monkey died six months after

Table 1. Summary of data on cross-protection

	Challenged with WNV		Challenged with JEV	
	Control (non-immune)	JEV immune	Control (non-immune)	WNV immune
1. Total number of monkeys	3	3	1	5
2. No. of monkeys which withstood challenge	1* (B26)	3	0	5
3. a) Clinical signs and symptoms (viral encephalitis)	2 (B20 B32)	0	1	2+ (B47 B57)
b) Death due to virus infection	2	0	1	0
c) Histopathological changes	2	0	1	0
d) Viraemia	2	1	1	0
e) Virus isolation from the CNS at necropsy	2	0	1	1** (Olfactory lobe) (B46)

*Monkey (B26) had N and HI antibodies in low titres, before challenge.
**Monkey (B46) exhibited no clinical signs and symptoms before and the time of death.
+Monkeys (B47 and B57) showed mild symptoms and recovered.

challenge without showing any symptoms of disease. The second monkey (No. B24) developed after 8 months progressive wasting disease resulting in loss of body weight. No virus was isolated from any of the organs of either monkey. The tissues examined did not reveal any evidence of viral encephalitis except of slight non-specific congestion and gliosis in the brain. The third monkey (No. B31) remained healthy for more than a year after virus challenge.

Prior to WNV challenge, the sera of all the three monkeys immunized with JEV developed HI antibodies to JEV and WNV (Fig. 1). After challenge, only one (B24) of the three monkeys showed higher titres (2-fold rise) of HI antibodies to JEV.

Monkeys challenged with JEV

The control JEV-infected monkey (No. B52) developed low-grade viraemia on day 5 p.i. followed by symptoms suggestive of viral encephalitis by day 14 as described by Ghosh *et al.* (1990). This monkey was sacrificed on day 17. The CNS showed pathological changes suggestive of viral encephalitis. JEV was

recovered from the brain and spinal cord of this monkey. Low titres of HI antibodies were detected in the serum collected on day 15 (Fig. 1).

One (No. B46) of the five WNV immunized monkeys challenged with JEV died on day 60 p.i. without showing any symptoms of disease. The monkey was

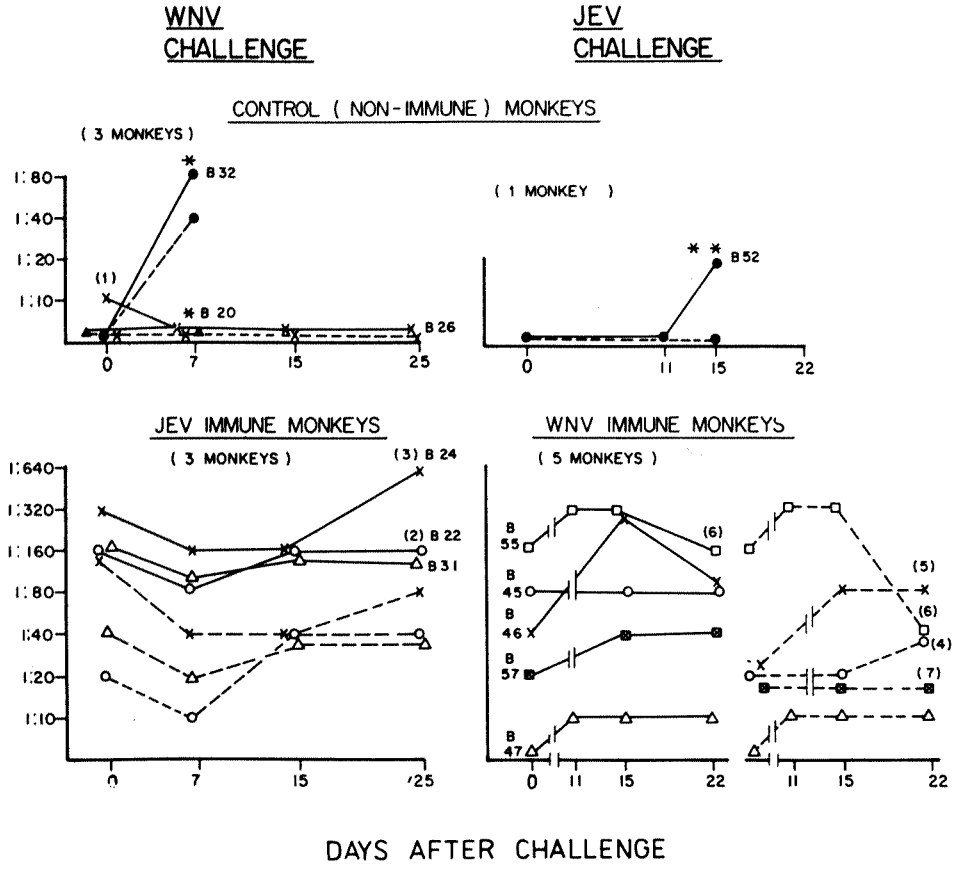

Fig. 1

HI antibody titres to JEV (—) and WNV (- - - -) in control and immune monkeys *Succumbed to infection on p.i. day 10. **Developed viral encephalitis on p.i. day 14 and necropsied on day 17. Few serum samples were also tested for N antibodies showed following titres to JEV - 1. Partially positive; 2. Pre-challenge 1 : 79 and on p.i. day 25 - 1 : 199; 3. Negative in pre-challenge sample and 1 : 158 on p.i. day 22, and against WNV - 4. Pre-challenge 1 : 501 and on p.i. day 22 - 1 : 1259; 5. Pre-challenge 1 : 1259 and on p.i. day 22 - 1 : 15848; 6. Pre-challenge 1 : 1000 and p.i. day 15 - 1 : 3981 (1 : 32 to JEV also); 7. Pre-challenge negative and on p.i. day 22 - 1 : 5.

autopsied and JEV was detected only in the olfactory lobe. Histopathological examination of the organs including olfactory lobe showed no changes suggestive of viral encephalitis except of non-specific congestion and gliosis. Of the remaining four monkeys two (Nos. B47 and B57) showed mild symptoms such as restricted movements, wrinkled face and slight shivering which subsided by day 20 p.i. The other two monkeys (Nos. B45 and B55) did not show any symptoms. None of the four surviving monkeys had virus in the blood. Two monkeys sacrificed on days 42 and 219 (B45 and B55, respectively) did not yield virus or showed any pathological changes.

HI and/or N antibodies to WNV and JEV were detected in four (B45, B46, B55 and B57) of the five monkeys immunized with WNV prior to JEV challenge (Fig. 1). After challenge all the four monkeys showed higher HI antibody titres (2-fold rise) to both the viruses. The fifth immunized monkey (B47) which had no antibodies to WNV and JEV prior to JEV challenge, showed low titres of HI and N antibodies to both the viruses after JEV challenge.

Discussion

Earlier studies on one-way cross-protection carried out by Price *et al.* (1963, 1967) on group B arboviruses have shown that immunization of spider-monkeys and chimpanzees with live Yellow fever, WN and formalin-inactivated Russian spring-summer encephalitis viruses cross-protected the animals against JE, WN, St. Louis, Murray Valley encephalitis or dengue (1, 2, 3 and 4) virus challenge. The route of virus inoculation employed in these studies was i.c. The peripheral route of challenge is been believed to be more suitable for such studies, being similar to the natural route of arboviral infections (Ghosh *et al.,* 1984, 1990). However, no clinical symptomps are encountered when adult rodents and non-human primates are inoculated experimentally with low doses of either JEV or WNV by intraperitoneal (i.p.) or s.c. routes. In the present study, therefore, intranasally-adapted mouse strains of both JE and WN viruses were used for the virus challenge by the i.n. route. The interaction of the virus with systemic immunity is more likely to occur by the i.n. route, compared to that by i.c. inoculation (Ghosh *et al.,* 1984).

Earlier the route of i.n. instillation has been employed successfully to confirm the protective effect of 6-MFA against JEV in the bonnet macaques (Ghosh *et al.,* 1990). The i.n. route not only offers a non-invasive challenge procedure but also produces clinical signs and pathological changes similar to those found in humans suffering from virus infection (Gopinath *et al.,* 1978; Ghosh *et al.,* 1984). We have, therefore, employed the same model for studying the two-way cross-protection between JEV and WNV. In the present studies it is observed that all the JEV immunized monkeys withstood WNV challenge, whereas WNV immunized monkeys showed lesser degree of protection to JEV challenge as evident from mild symptoms of encephalitis followed by recovery

in 2 WNV immunized monkeys and isolation of virus from the olfactory lobe of the third monkey. Interestingly, two each of the five WNV and three JEV immunized monkeys were cross-protected inspite of the absence of detectable N antibodies; whereas one of the control monkeys having low N antibodies to JEV prior to challenge also withstood challenge with JEV. These observations when considered together with mild clinical symptoms presented by 2 of the 5 WNV immunized monkeys and virus isolation from olfactory lobe of third monkey upon JEV challenge, indicate that immunization with WNV could only reduce the severity of disease. This probably might explain the occurrence of human arboviral encephalitis outbreak in India, which are mainly due to JEV inspite of the widespread WNV activity; WNV has been isolated only rarely from the brains of human fatal encephalitis cases (George *et al.*, 1984). The present animal-model employing bonnet macaques may be useful for assessing the potency of vaccines, antiviral drugs and for the studies on the cross-protection arising out of the infections due to the serologically-related viruses in nature.

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