Arch Virol (1997) 142: 1937–1952

Archives of Virology

Isolation of a measles virus variant: protection of newborn mice from measles encephalitis by 24 h prior intracerebral inoculation with the variant

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Accepted June 28, 1997

Summary. A small plaque mutant with reduced neurovirulence in newborn mice was obtained from Edmonston strain measles virus after propagation for 5 months in NIH3T3 cells. It retained the antigenicity of the parental virus and tended to induce higher neutralizing antibody titers in the adult BALB/c mice. The intracerebral (but not intraperitoneal) inoculation of the live mutant virus one day before prevented the newborn BALB/c mice from encephalitis caused by the intracerebral challenge with the parental strain at a dose of 10–20 LD_{50}. The intracerebral inoculation with the mutant virus whose replication capacity was inactivated by UV-irradiation was ineffective. The protection was not attributed to interferons nor to viral interference. The mechanism remains unknown.

Introduction

Propagation of virus in an unnatural host sometimes results in isolation of viruses with altered characters [13–15, 17–19, 21, 24, 27, 34, 35]. In this paper, we describe a novel mutant measles virus derived from a mouse cell line persistently infected with the strain, Edmonston. It produced small plaques on Vero cells and had increased plating efficiency in mouse cells. The intraperitoneal injection of the virus induced neutralizing antibody in mice as high as or even higher than its parental strain. When inoculated intracerebrally into newborn mice, the mutant showed non neurovirulence while the parental strain did so. Curiously, the intracerebral inoculation of the mutant virus one day before the intracerebral challenge with the parental type Edmonston strain protected the newborn mice from the encephalitis caused by the latter.

Materials and methods

Virus and cells

Edmonston strain of measles virus (Ed-MV) was obtained from American Type Culture Collection (ATCC). Encephalomyocarditis virus (EMCV) was the strain maintained in National Institute of Health Japan for the IFN potency test. Vero cells were obtained from Dr. F. Kobune, National Institute of Health, Japan. L cells used for IFN assay were the WHO standard cells. NIH3T3 cells were obtained from ATCC and maintained in our laboratory. The culture medium employed was DMEM supplemented with 7% fetal calf serum.

Virus assay

The viruses were assayed as described previously [35]. Minor modifications are described in the text. The UV-Vero assay was devised by our group [27]. The procedure was similar to the UV-XC assay of mouse leukemia viruses [26] i.e., the mouse cells showing no cytopathogenicity after measles virus infection were irradiated with UV at a dose of 500 ergs/mm^2, and overlaid with Vero cells. The plaques were counted 24 h later. It detected only the infected cells and not the virus adsorbed to the cells [27].

Neutralization assay

Measles virus was diluted to a concentration of 1,000 p.f.u./ml. Five hundred µl of the virus were mixed with the some amount of the serum which had been diluted serially 2-fold, and incubated for 1 h at 29 °C. Two hundred µl aliquot of the virus serum mixture was inoculated to each well of 12-well plates which had been plated with 5×10^5 Vero cells per well. After adsorption for 1 h, the inoculum was removed and the agar medium was overlaid. The plaques were counted 4–5 days later for Ed-MV and 7–8 days later for MVp.

Assay of IFN

The 10% cerebral homogenates in DMEM were centrifuged at 10,000 rpm for 10 min, and treated with anti-MV serum at a final concentration of 250 measles virus neutralizing titer. The L cells plated in 96-well plates were incubated with the cerebral homogenates or with the standard IFN (WHO standard IFN 100 IU/ml) for 24 h. Then each well was inoculated with 10^3 PFU of EMCV, and 24 h later the cultures were fixed with 10 % formaldehyde and stained. The titer was expressed in laboratory unit (L.U.); 128 L.U. corresponded 50 IU of WHO standard β IFN.

Mice

Pregnant SPF mice 1 or 2 days before delivery were purchased from Nihon SLC, Hamamatsu, and newborns were used for testing the neurovirulence of measles virus. The mouse strains were inbred C3H/He, BALB/c, C57BL/6, ddY and 1CR. Each litter of mice with their mother was kept in a separate cage. Three to four week old BALB/c female mice used for testing the antibody response were also purchased form Nihon SLC, Hamamatsu. Four to five mice were kept in a cage. The intracerebral injection for testing the neurovirulence and the intraperitoneal injection for testing the antibody response were performed as recommended in a manual [11]. The experiments were conducted in an air conditioned P2 animal room (room temperature was kept around 20 °C) in our laboratory.

Pathological examinations

The organs were fixed in 10% buffered formalin solution for 5 days and embedded in paraffin. Serial sections were used for hematoxylin and eosin staining for histological examination and also used for antigen detection by ABC immunoperoxidase method using a monospecific rabbit antibody against measles virus NP protein.

Results

Persistent infection of Edmonston measles virus in mouse cells

NIH3T3 cells plated in an amount of 10^6 cells per 260 ml flask were infected with 10^6 p.f.u. of Edmonston strain measles virus (Ed-MV). After adsorption, the cells were cultured in 10 ml of the medium. The cells were subcultured at 3 to 4 day intervals. No cytopathic changes were detected at any stages of the infection. The whole culture infected for 23 days was harvested for the plaque assay in Vero cells. The titer was about 2×10^5 p.f.u./m.l, which was retained for at least five months. When Vero cells were infected with Ed-MV at a similar m.o.i., the titer usually went up to 10^6 to 10^7 p.f.u./ml 4–5 days post-infection and then the cultures were lost due to cytopathogenicity of the virus.

In order to know the mode of the persistence of Ed-MV in the infected NIH3T3 cells, the following experiments were performed. The cells infected for 155 days were suspended, diluted and inoculated into 96 well plates in an amount of 1.6 cells per well in average. After 10 days, wells with growing cells were scored. In 192 wells, only sixteen wells were positive for the cell growth. Only two of the sixteen wells with viable cells positive for virus production. Further sub-cloning of the two clones could not establish the cell lines stably producing Ed-MV. The cells were also cultured for 8 days in the medium containing various concentrations of an SSPE patient's serum which was known to neutralize, Ed-MV [35], and cultured further for 19 days without the antiserum. The medium was changed, and 24 h later the whole cultures were assayed for the viral infectivity in Vero cells. The infected cells which had been cultured in the medium containing 0.2 to 5% antiserum failed to produce plaques, while the control cultures continued to produce the infectious virus. Thus, the Ed-MV-infected NIH3T3 cell cultures were considered to be virus-carrier cultures; probably, the infected cells which finally died transmitted the virus to the uninfected neighbouring cells before death.

Plaque formation and plating efficiency of the measles virus recovered from carrier NIH3T3 cell

Figure 1 compares the plaques produced in Vero cells by the virus released from the NIH3T3 cells infected for five months (MVpi) with those produced by the parental Ed-MV. The plaques produced by the former (0.08 ± 0.04 mm) was about one third in diameter of those produced by the latter (0.23 ± 0.08 mm). It should be noted that the plaques produced by MVpi were all small-sized, and large plaques which were produced by the wild type were undetectable.

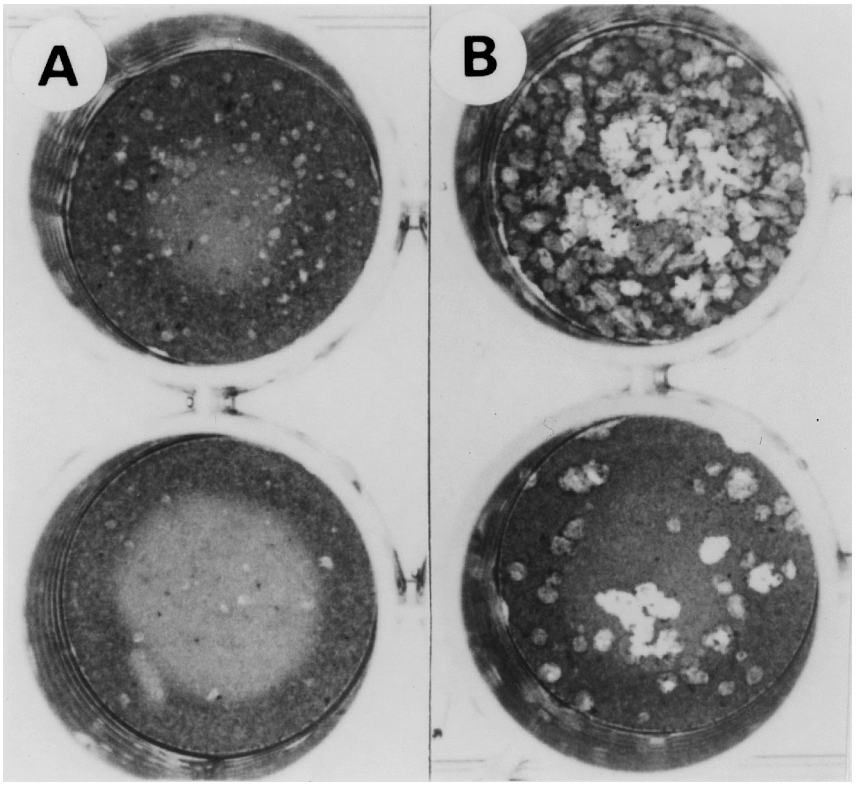

Fig. 1. Plaques produced by Ed-MV and those produced by MVpi, the virus obtained after the long-term propagation of Ed-MV in the NIH3T3 cell. Vero cell monolayers were infected with Ed-MV (**B**) or MVp (**A**). The upper dishes were inoculated with 1,000 p.f.u. / dish and the lower ones with 100 p.f.u. / dish. After adsorption, the monolayers were cultured under agar medium. The cultures were fixed with formalin 3 days post infection

The plating efficiencies in NIH3T3 cells of MVpi and the parental Ed-MV were compared. NIH3T3 cells were plated in 12 multi-well plates in an amount of 5×10^4 cells / well and Vero cells in an amount of 1×10^6 cells / well. After 2 h adsorption, the plates were washed with the phosphate buffered saline (PBS) three times, and cultured. The NIH3T3 cells infected with either virus

Table 1. Plaquing efficiency on mouse NIH3T3 cells relative to that on Vero cells

	Ed-MV	MVpi[b]	MVp[b]
NIH3T3/Vero[a]	0.8	56.8	82.1

[a] [p.f.u. in NIH3T3 cells / p.f.u. in Vero cells] $\times 100$. The p.f.u. in NIH3T3 cells was obtained by the UV-Vero assay

[b] MVpi was the virus produced by the NIH3T3 cells infected with Ed-MV for five months, and MVp was the clone obtained from MVpi after two successive plaque purifications

were submitted to the UV-Vero assay [27] four days after infection. The cell were fixed on the following day. As shown in Table 1, the parental Ed-MV plated on NIH3T3 cells plated about 100-fold less efficiently than on Vero cells, while MVpi plated on NIH3T3 cells nearly as efficiently as on Vero cells (57%).

Plaque purification of MVpi

For further characterization of MVpi, the plaque purification was performed. NIH3T3 cells were plated in 12 well plates in an amount of 5×10^4 cells /well, and infected with MVpi which had been diluted serially 10-fold. Three days later, the cells were submitted to the UV-Vero assay. The medium was removed on the next day and 1% agarose medium was overlaid onto the cells. Plaques were chosen from the plates developing less than five plaques, picked, and inoculated into NIH3T3 cells for virus propagation. The plaque purification was repeated once more. One virus clone thus obtained was coded as MVp. Mvp was then passaged serially in Vero cells. After four serial passages in Vero cells, the plaque size in Vero cells and the plating efficiency in NIH3T3 cells of this virus were compared with those of Ed-MV and MVpi. The character of the small sized plaque (0.08 ± 0.04 mm in diameter in contrast to Ed-MV whose plaque diameter was 0.23 ± 0.08 mm) and the high plating efficiency in NIH3T3 cells (Table 1) remained unchanged. The small plaque of MVp was not due to the temperature sensitiveness, because the plaques produced by MVpi at 30 °C were even smaller than those produced at 37 °C.

Neutralization kinetics

We compared the neutralization kinetics of MVp and Ed-MV by using the polyclonal rabbit anti-Ed-MV serum (kindly provided by Dr. Kobune, National Institute of Health, Japan). The neutralization kinetics was identical for the two viruses (Fig. 3), indicating that the MVp had the similar epitope(s) (if not identical) for the neutralizing antibody.

The antibody inducing capacity was also compared. The 4-week old BALB/c female mice were injected intraperitoneally (i.p.) with Ed-MV or MVp at a dose of 10^5 p.f.u. The serum was obtained 6 or 8 weeks after injection. Six weeks after the injection, a group of mice received a booster i.p. injection with the same dose of the virus and the serum was obtained two weeks later. Each serum sample was pooled from three mice. At 6 weeks after the single injection, neutralizing antibody titer (concentration of the serum which reduced the plaque number by 50%, ND_{50}) was 10–20 for Ed-MV-immunized mouse sera while 20–40 for MVp-immunized mouse sera. At 8 weeks, without the second shot, the ND_{50} was 10 for Ed-MV-immunized mouse serum while 80 for MVp-immunized mouse sera. At 8 weeks, with the second shot at 6 weeks, the ND_{50} was 40–80 for Ed-MV-immunized mouse sera while much higher than 160 for MVp-immunized mouse sera. MVp always induced a higher titer of neutralizing antibody than Ed-MV.

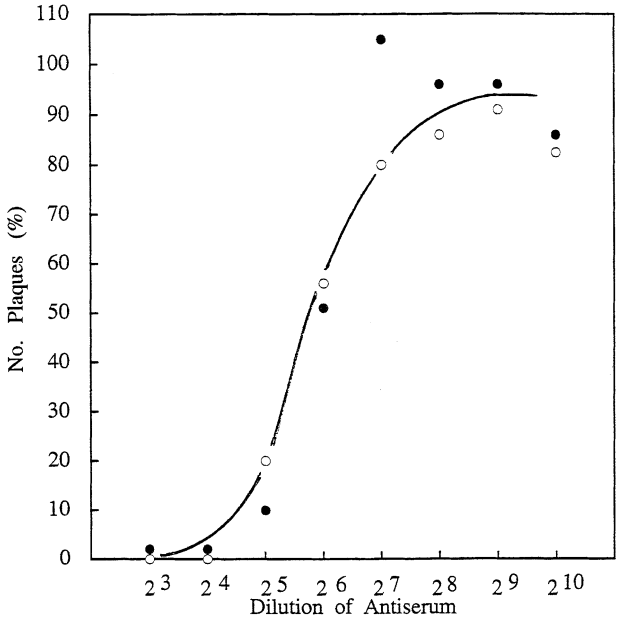

Fig. 2. Neutralization assay. The vertical axis shows % control of number of plaques per plate and the horizontal axis shows the dilution of the serum. ○ Ed-MV; ● MVp

Pathogenicity to newborn mice

In order to compare the neurovirulence [2, 4, 10, 12, 20, 22] of MVp and Ed-MV, the newborn BALB/c mice (48 h after birth) were inoculated intracerebrally (i.c.) with these viruses at doses of 10^4 and 5×10^3 p.f.u. in 10 µl, respectively. Up to ten days, the mice in the two groups were apparently indistinguishable, but from 10 days, a population of the Ed-MV-inoculated mice began to show illness characterized by ruffled fur, hunched posture, hyperexcitability and tremor of the tail [8, 16, 25], and some of them died. The mice inoculated i.c. with MVp all grew normally. The intraperitoneal injection of either Ed-MV or MVp in the newborn BALB/c mice induced neither neurological signs nor growth retardation as already reported [22]. Ed-MV and MVp were injected i.c. to newborns for comparison of neurovirulence in various mouse strains (Exp #1, Table 2). C3H/He was the most sensitive to Ed-MV in accordance with the previous report [22] and ICR was the most resistant. MVp failed to induce neurological changes even in C3H/He. The virus dose which killed 50% of newborn BALB/c mice, LD_{50}, was calculated according to Reed and Muench [1] using the data of Exp. #2 in Table 2. It was about 5×10^2 p.f.u. for Ed-MV.

Protection of mice from Ed-MV induced killing by previous i.c. inoculation of MVp

We asked weather the previous i.c. inoculation of the mice with MVp might prevent the killing by Ed-MV. On the next day of birth, group A consisting of 7

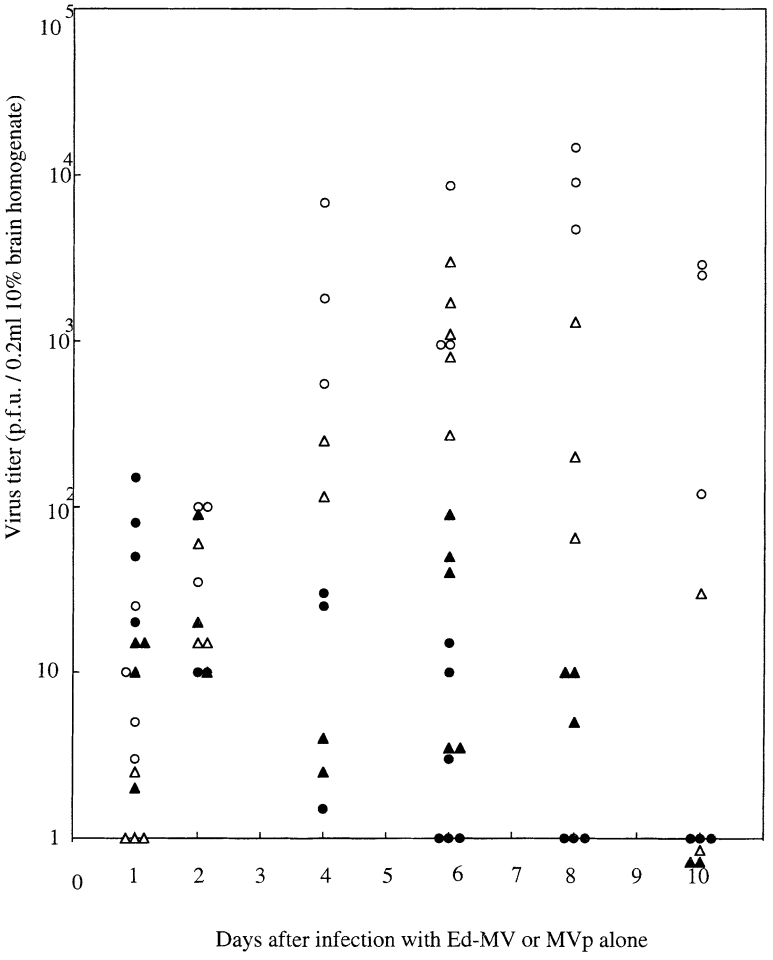

Fig. 3. Replication of measles virus in the mouse brain. Two litters of newborn BALB/c mice (24 h after birth) were inoculated i.c. MVp at a dose of 10^4 pfu/10 µl and another litter of the mice with the culture medium (10 µl). On the next day, one of the two litters of mice inoculated with MVp and the mice inoculated with the medium was inoculated with Ed-MV at a dose of 5×10^3 pfu/5 µl. The mice were sacrificed at intervals to make a 10% brain homogenate in DMEM. The homogenates were freeze-thawed, and centrifuged at 1000 rpm for 10 min. The supernatant was serially diluted and inoculated to the Vero cells in 12 well plates in a volume of 0.2 ml/well. Open circles: mice inoculated i.c. with Ed-MV only. Triangles: mice inoculated with MVp and then with Ed-MV (the open symbols indicate titer of Ed-MV and the closed symbols indicate that of MVp; Ed-MV and MVp could be distinguished by the different plaque sizes). Closed circles: mice inoculated i.c. with MVp only. The vertical axis indicates virus titer per 0.2 ml of the 10% homogenate. The horizontal axis indicates days after injection with Ed-MV (both for Ed-MV alone or for MVp followed by Ed-MV) or with MVp alone

newborns and group B consisting of 6 newborns were inoculated i.c. with 20 µl culture medium, and group C consisting of 7 newborns and group D consisting of 6 newborns were inoculated i.c. with MVp at dose of 10^4 p.f.u. in 20 µl; all the mice received injection on the right-side brain. On the next day, all the mice

Table 2. Neurovirulence of Ed-MV and MVp in newborns of various mouse strains

Exp.	Inoculum		Mouse strains	Total	Died	%
#1	Ed-MV	5×10^3 p.f.u.	C3H/He	12	12	100
			BALB/c	16	14	87
			C57BL/6	16	5	31
			ddY	23	9	39
			ICR	22	1	5
	MVp	1×10^4 p.f.u.	C3H/He	7	0	0
			BALB/c	8	0	0
			C57BL/6	13	0	0
			ddY	18	0	0
			ICR	15	0	0
#2	Ed-MV	2.5×10^4 p.f.u.	BALB/c	18	18	100
		5×10^3		16	15	93
		1×10^3		19	14	73
		2×10^2		13	4	31

received i.c. injection of 5×10^3 p.f.u. Ed-MV (in 5 μl), group A and C on the right side of the brain and group B and D on the left side. In 10 weeks observation, 5 in 7 in group A and 4 in 6 group B mice died (mortality about 70%), while in group D none of 6 mice died (mortality 0%) and in group C only 2 in 7 died (mortality about 30%); the mice which survived were completely healthy (Table 3, Exp. #1). The experiment clearly showed that the prior i.c. inoculation with MVp on the same or opposite side of the brain protected the mice from killing by i.c. inoculation with Ed-MV. The phenomenon was highly reproducible (Exp. #2, #3 and #4 in Table 3). In the above experiments 5×10^3 and 1×10^4 p.f.u. Ed-MV challenge doses corresponded about 10 and 20 LD_{50}, respectively since one LD_{50} was about 5×10^2 p.f.u.

The histopathological and immunohistochemical examinations were performed. In the brains of the mice inoculated with 1×10^4 p.f.u. Ed-MV for 7 days, pictures of meningitis, small hemorrhages and distribution of small foci of neuronal degeneration in cortex were found. The measles virus antigens were detected in the endothelial cells of small veins or capillaries, and in some neurons. The moribund or died mice on day 14 to 16 showed severe damage with large amount deposition of viral antigens in cortical neurons. Overall 15 in 20 mice showed such pathological changes. In the mice inoculated with 4×10^4 p.f.u. MVp, there were no obvious pathological changes, though one mouse among 20 had small lesions with antigen in cerebral cortex. Among the mice which received injection of 4×10^4 p.f.u. MVp before challenge with 1×10^4 p.f.u. Ed-MV, three mice showed pathological changes indistinguishable from those infected with Ed-MV alone (at days 7 and 13) but the remaining 24 mice had normal histological pictures. No pathological changes characteristic to this group were detected.

Table 3. Protection of mice from Ed-MV-induced killing by previous inoculation with MVp

Experiment	Group	First inoculation			Second inoculation		Number of mice		
		virus	dose	site	virus	dose	total	died	(%)
#1	A	m[a]		s[c]	Ed	5×10^3	7	5	(71)
	B	m		o[d]	Ed	5×10^3	6	4	(67)
	C	MVp	1×10^4	s	Ed	5×10^3	7	2	(29)
	D	MVp	1×10^4	o	Ed	5×10^3	6	0	(0)
#2		m		s	Ed	5×10^3	9	9	(100)
		MVp	1×10^4	s	Ed	5×10^3	5	0	(0)
#3		–[b]			Ed	5×10^3	6	4	(67)
		MVp	4×10^4	s	Ed	5×10^3	5	0	(0)
		–			Ed	1×10^4	12	10	(83)
		MVp	4×10^4	s	Ed	1×10^4	9	1	(11)
#4		MVp			–		13	0	(0)
		–			Ed	5×10^3	6	6	(100)
		MVp	4×10^4	s	Ed	5×10^3	6	1	(17)
		–			Ed	1×10^4	6	6	(100)
		MVp	4×10^4	s	Ed	1×10^4	7	1	(14)
#5		–			Ed	1×10^4	5	4	(80)
		MVp	4×10^4	s	Ed	1×10^4	9	1	(11)
		MVp	UV-killed	s	Ed	1×10^4	9	6	(67)
#6		m		s	Ed	5×10^3	7	7	(100)
		MVp	1×10^4	s	Ed	5×10^3	12	4	(33)
		MVp	UV-killed	s	Ed	5×10^3	14	14	(100)
#7		–			Ed	5×10^3	7	7	(100)
		–			$\left\{\begin{matrix}\text{Ed} \\ \text{MVp}\end{matrix}\right.$ + $\left\{\begin{matrix}1 \times 10^4 \\ 4 \times 10^4\end{matrix}\right.$		14	14	(100)
		MVp	1×10^4	s	Ed	1×10^4	8	2	(25)
#8		–			Ed	5×10^3	5	5	(100)
		MVp	4×10^4	i.p.[e]	Ed	5×10^3	15	13	(87)
		MVp	1×10^4	s	Ed	5×10^3	7	2	(27)

[a] Medium
[b] Untreated
[c] First and second inoculations were on the same side of the brain
[d] First and second inoculations were on the opposite side of the brain
[e] Inpraperitoneal injection

Simultaneous inoculation of MVp with Ed-MV (Exp #7, Table 3) or i.p. inoculation before Ed-MV challenge (Exp #8, Table 3) did not show significant protective effect, Ultraviolet (UV) irradiation ($\lambda = 2537$ Å) at a dose (about 7.5×10^3 ergs/mm^2) which reduced the infectivity by 10^4-fold, i.e., at a lethal dose for the virus infectivity, completely abolished the resistance-inducing capacity of MVp (Exp. #5 and #6 in Table 3).

Replication of the virus in the brain

The newborn mice were divided into three groups. One group received i.c. injection of Ed-MV (5×10^3 p.f.u.) 2 days after birth, another group received i.c. injection of MVp (1×10^4 p.f.u.) on the next day of birth, and the third group received i.c. injection of MVp on the next day of birth and then i.c. injection of Ed-MV on the following day. As shown in Fig. 3, MVp at low titers was detected only transiently for the first 6 days and became undetectable later than 8 days. Ed-MV replicated to attain much higher titers and persisted till ten days at least. In the mice which received i.c. injection of MVp before Ed-MV challenge, the titer of Ed-MV rose initially for 6 days (though to a slightly lower titer) and then declined. This observation indicated that the complete virus resistance was not established in the brain 24 h after the i.c. injection with MVp.

Interferons in the infected brain

Some of the samples used for the virus assay in the previous section were used for estimation of interferons (IFNs). Before the assay, the measles virus in the homogenate was neutralized with the anti-measles virus antibody. The interferon was assayed by the standard method using encephalomyocarditis virus (EMCV) and L cells. The IFN was not detected in MVp-infected mouse brains during the observation. In Ed-Mv-infected mouse brains, IFN became detectable from 6 days. In the mice which received MVp injection and then Ed-MV injection on the following day, IFN was detected transiently on days 6 and 8 (Fig. 4A). When the Ed-MV virus titer was plotted against IFN activity, a proportional relation was observed (Fig. 4B). This observation suggested that the IFN was not a major factor responsible for the protection of Ed-MV by MVp; it rather reflected the replication of the parental Ed-MV.

Protection of mice from SSPE-induced lethality

The 4-week BALB/c female mice were inoculated i.c. with 1.2×10^5 p.f.u. MVp and then inoculated with SSPE carrier human embryo fibroblastic cells equivalent to 600 p.f.u. or 140 p.f.u. (see [31] for details of the SSPE and a definition of p.f.u. of SSPE) on the following day. As a control, the uninoculated BALB/c female mice were inoculated with the same dose of SSPE cells. The prior i.c. inoculation of MVp partially protected the mice from the killing by the SSPE (Exp #1, Table 4).

Protection of mice from EMCV-induced lethality

We also asked whether the protection from viral encephalitis by MVp was specific to measles virus or not. A group of BALB/c mice were inoculated i.c. with MVp (4×10^4 p.f.u.) on the next day of birth and then i.c. with EMCV (1–10 p.f.u.) on the following day; the other group of the newborns were inoculated with EMCV only. By the prior i.c. inoculation of MVp, the time of killing was delayed by 1–2 days and the mortality was even reduced (Exp #2, Table 4).

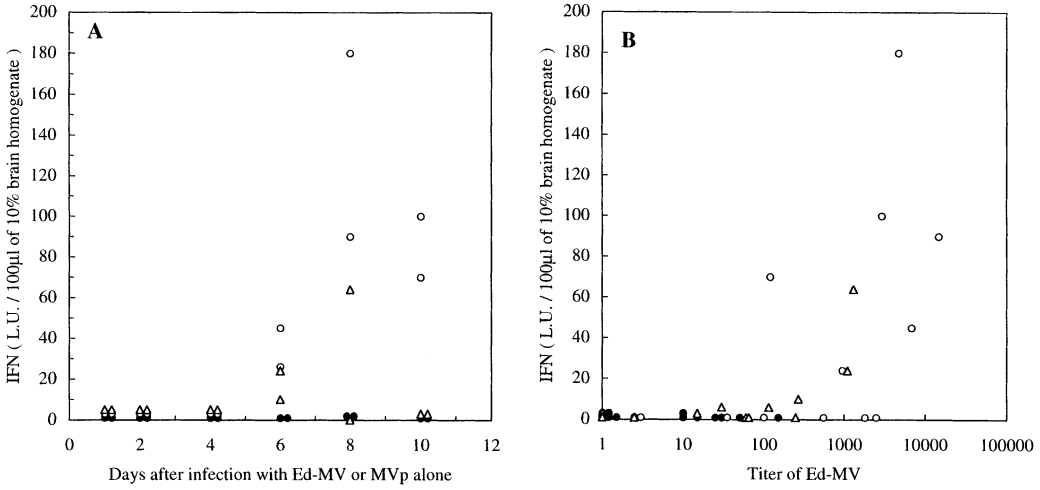

Fig. 4. Interferon activity in the brains of the mice inoculated i.c. with Ed-MV, with MVp or with MVp and then Ed-MV. The brain homogenates used in Fig. 3 were used for titration of IFN. **A** IFN titer as a function of days post-infection. All the samples were neutralized with anti-measles virus antiserum before assay. The IFN assay was performed under the standard condition using L cells and EMC virus. The vertical axis indicates IFN L. U. (laboratory unit) / 100 µl (50 IU of the WHO standard IFN β corresponds 128 L.U.) The horizontal axis indicates days after infection (see legend to Fig. 3). **B** IFN titer as a function of titer of Ed-MV. The virus titer for mice inoculated with MVp and then Ed-MV is that of Ed-MV. Each point represent the data of one newborn mouse. Closed circles: mice infected with MVp. Open circles: mice infected with Ed-MV. Open triangles: mice infected with MVp and then Ed-MV on the following day

Table 4. Protection of mice from SSPE or EMCV-induced killing by previous inoculation with MVp

Experiment	Animal	First inoculation			Second inoculation		Number of mice		
		virus	dose[a]	site	virus	dose[a]	total	died	(%)
#1	4-wk old	–			SSPE	600	10	10	(100)
	BALB/c	MVp	1.2×10^5	i.c.	SSPE	600	11	7	(63)
		–			SSPE	140	10	9	(90)
		MVp	1.2×10^5	i.c.	SSPE	140	11	5	(45)
#2	newborn	–			EMCV	1	23	12	(52)
	BALB/c	MVp	4×10^4	i.c.	EMCV	1	23	5	(21)
		–			EMCV	5	15	13	(86)
		MVp	4×10^5	i.c.	EMCV	5	15	8	(53)
		–			EMCV	10	8	8	(100)
		MVp	4×10^4	i.c.	EMCV	10	8	7	(87)

[a]p.f.u. or p.f.u. equivalent SSPE cells

Discussion

We isolated a mutant measles virus coded as MVp after long-term culture of Edmonston strain measles virus (Ed-MV) in NIH3T3 cells. The mutant was characterized by small plaque in Vero cells and relatively high plating efficiency in the mouse NIH3T3 cells. After five months of culture in NIH3T3 cells, all the progeny viruses showed the small plaque mutant phenotype. This near complete conversion of the virus population was probably because the mutant had replication advantage over the parental type in mouse cells (Table 1). The mutant had no detectable neurovirulence when it was injected i.c. into newborn BALB/c mice, but retained the antigenicity of the parental Ed-MV. When the adult mice were inoculated with the same infectious dose of the virus, MVp tended to induce higher antibody titer than Ed-MV. The observed higher antibody titer induced by MVp was probably not due to its higher capacity of replication in the mice; MVp replicated rather poorly in vivo.

Interestingly, if the mice were inoculated i.c. with MVp one day before i.c. inoculation with Ed-MV, the mice were protected from the encephalitis caused by Ed-MV. The previous i.p. inoculation with MVp was not protective, however; processes leading to the protection are considered to take place only locally in the brain. As the inoculation of MVp on the opposite cerebral hemisphere of the side of Ed-MV challenge protected the mice from the lethality caused by the latter, the resistance state induced by MVp appeared to spread quickly in the brain in the following 24 h. The phenomenon was examined from various points. First, we tried to reproduce the phenomenon in culture. NIH3T3 cells (10^5 cells/60-mm Petri dish) were infected with MVp at a dose of 4×10^4 p.f.u./dish, and on the following day, the cultures were challenged with Ed-MV at a dose of 5×10^3/dish. The replication of Ed-MV was unaffected by the previous infection with MVp (data not shown). Therefore MVp did not interfere with the Ed-MV in vitro. (It should be mentioned here, however, that, when the MVp carrier NIH3T3 cells and the control NIH3T3 cells were infected with Ed-MV at an m.o.i. of 1, Ed-MV failed to replicate almost at all in the former while the titer went up to 1×10^6/ml in the latter. Here, IFN activity was not detected in the supernatant of the MVp carrier cells. The interference could be due to a kind of the viral interference or down regulation of receptor [28]). Second, we examined if the antigenicity of the virus alone was enough for inducing the protective state. When MVp was irradiated with UV ($\lambda = 2537$ Å) at a dose which was just enough for abolishing the infectivity (about 7.5×10^3 ergs / mm^2), MVp lost the protective effect. As the inactivation of viral protein function required far higher doses, for example, inactivation of HA activity of influenzavirus required about 10^{10} fold higher doses than that required for inactivation of infectivity [33], probably the antigenic stimulation alone was not enough, and the MVp had to replicate. Third, we examined the role of IFN in this phenomenon. The measles virus was actually very sensitive to IFN α, β and γ. However, in the brain of mice inoculated with MVp, we failed to detect the IFN activity. The IFN activity in

the brain of the infected mice was rather proportional to the replication of the Ed-MV. It is nevertheless possible that the locally produced minute amount of IFNs [7] may be protecting the mice from Ed-MV. However, the replication of MVp was only marginal in the brain and yet the resistant state spread quickly to the other side of the brain. In addition, the near complete suppression of the encephalitis is difficultly explained by the less than one order reduction of the virus replication (see virus titers on day 6 in Fig. 3). Thus, even the role of locally produced IFNs may be very small, if not entirely excluded, though in the adult mice involvement of IFN γ in antiviral defence against encephalitis caused by special strains of measles virus was reported [7]. We also asked induction of putative inhibitors of Ed-MV infection after MVp inoculation in the brain. The Vero cells were treated for 24 h with the serially diluted brain homogenate and then infected with Ed-MV. Though the homogenate was slightly inhibitory on Ed-MV infection (data not shown), the inhibitory activity was not specifically augmented by the MVp infection.

When the virus replication was followed in the brain of the mice inoculated first with MVp and then Ed-MV on the following day, the replication of Ed-MV was hardly inhibited initially. The appreciable decline of the replication was observed only from day 8 to 10. This indicates that, 24 h after inoculation with MVp when Ed-MV was injected, mouse brain was actually susceptible to Ed-MV infection. As the simultaneous infection of MVp with Ed-MV did not protect mice from the killing by Ed-MV, some event which would play a critical role in disease protection must have occurred in the 24 h after MVp injection. It should be mentioned that the susceptibility of the newborn mice rapidly declined from 3 to 4 days after birth. In the condition where 100% of the BALB/c newborns infected with Ed-MV at 1 day after birth were killed, 62% of the mice at 3 days and only 30% of the mice at 4 days were killed. Therefore, a slight delay of Ed-MV induced pathogenesis could result in the prevention of encephalitis caused by Ed-MV.

Schröder et al. reported that, in herpes simplex virus (HSV) infection in the adult mice, co-infection with non-encephalitogenic strain protected mice against lethal encephalitis by a virulent strain [30]. The observation in their report overlaps with ours in that the protection state was established locally very rapidly. However, in HSV case, the protection could be obtained even when the non-encephalitogenic virus was injected 7 days after infection with the virulent virus and the replication of the virulent virus was blocked at the initial stage. In addition, non-replicating or UV-killed non-encephalitogenic virions could induce the resistant state. Ohuchi et al. reported that Ed-MV protected the adult mice from lethal encephalitis caused by i.c. inoculation of the cells carrying Biken strain of subacute sclerosing panencephalitis (SSPE) virus [23]. Their observation was quite similar to Schröder et al.'s [30], and inoculation with Ed-MV after SSPE infection was protective. Involvement of IFN and T lymphocytes was suggested here. MVp was found protective also against SSPE. But, our observation appears different from Ohuchi et al.'s [23] in various respects, e.g. age of the mice suitable for the experiments, timing of

inoculation of attenuated strains for obtaining protection from the virulent strain, etc. Taguchi et al. [32] reported a similar rapid induction of the resistance against a virulent mouse hepatitis virus by prior injection of low virulent strain as measured by the mortality and virus replication. Their experiments suggested an involvement of the peritoneal macrophages, but the mechanism has remained unclear. It is possible that in our case brain macrophages were involved in the MV-induced resistance against the measles virus encephalitis. As MVp was partially protective to EMCV-induced encephalitis, the protective effect of MVp against Ed-MV-induced encephalitis may be a non-specific protection against viral encephalitis, of wider range. So far, the mechanism of this phenomenon has remained a mystery, but the system will be a useful tool for analysis of viral encephalitis.

Acknowledgement

Our thanks are due to Dr. F. Kobune, National Institute of Infectious Diseases, for providing us with the antisera and for his valuable advice.

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Received April 9, 1997