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A novel variant of Sindbis virus is both neurovirulent and neuroinvasive in adult mice

S. Lustig, M. Halevy, D. Ben-Nathan, and **Y. Akov**

Department of Virology, Israel Institute for Biological Research, Ness Ziona, Israel

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Summary. A strain of Sindbis virus (SV), recently isolated from mosquitoes in Israel, was used as a source for variants which differ in neuroinvasiveness and virulence that were generated by serial passage of SV in suckling and weanling mouse brain. At the 15th passage a neurovirulent variant was observed and designated SVN (neurovirulent). After 7 more passages in weanling mouse brains, another variant was observed and designated SVNI (neuroinvasive) and both were isolated and purified. All strains caused similar viremia after intraperitoneal (I.P.) injection of weanling mice, but whereas SV was neuroinvasive but nonvirulent, SVN was neurovirulent but noninvasive and SVNI was both virulent and invasive. SVNI is the first SV variant which is both neurovirulent and neuroinvasive in weanling mice. Co-injection I.P. of SV + SVN resulted in presence of SV alone in the mouse brain; co-injection of SVNI + SVN resulted in full-titered replication of both strains in the brain. We assume that this is achieved through a breach of the blood brain barrier effected by SVNI replication and used by SVN for co-invasion. SV probably invades the brain by a different mechanism. I.P. infection with SVNI of inbred BALB/c mice gave rise to clinical signs only in a few mice even though substantial viremia was demonstrated.

Introduction

Sindbis virus (SV) is the type species of alphavirus, a large group of viruses which cause diseases in humans (and other species) – from rash and arthritis to acute encephalitis. SV (strain AR339) was isolated in 1953 in Egypt [21] and since then has been the subject of numerous studies concerning its biological activity and chemical composition [6, 7]. SV is among the least pathogenic of the alphaviruses and is lethal only in suckling mice. It replicates in muscle cells causing myositis, followed by viremia which spreads the virus to the central nervous systems (CNS) to cause an encephalitis which kills suckling mice but

is non-fatal to weanlings. The SV genome has been sequenced [20]. SV has been used, together with variants in virulence, to study the molecular basis of neurovirulence [9, 15].

The present study uses a strain of SV which was isolated by Dr. J. Peleg from a pool of culicine mosquitoes caught in southern Israel in 1983, i.e., 30 years after AR339. The aim of this study was to develop variants of SV by consecutive passages in the brain of suckling and weanling mice and to compare the biological characters of SV (Peleg) and its variants to those of SV (AR339) and its variants, as they are reported in the literature [7]. We present evidence that we have succeeded in isolating two variants – one of them neurovirulent and another is both neurovirulent and neuroinvasive.

Materials and methods

Viruses

A Sindbis virus strain derived from AR339 has been in our laboratory for many years and it is lethal to weanling mice by I.C. injection. It was designated SVN-NZ (Sindbis virus, neurovirulent, Ness-Ziona). Its exact history is unknown but it had many passages in mouse brain, chick fibroblasts and BHK cells. The more recent Sindbis virus isolate was received from Dr. J. Peleg (formerly of our institute), who isolated it in 1983 from a pool of culicine mosquitoes gathered in southern Israel. The source and history of Semliki Forest virus (SFV) and West Nile virus (WNV) have been reported [1].

Mice

Outbred ICR mice (CD1) were received form Charles River, U.K. Outbred Webster Swiss (DF1), and inbred BALB/cByJIco were purchased form IFFA CREDO, France. Suckling mice were used at 2–4 days of age and weanling mice were used when 4 weeks old.

Immune sera

Standard rabbit anti-SV (AR339) immune serum was received from the NIH. Rabbit anti SVN-NZ immune serum was prepared by three I.P. injections into rabbits of 10^8 PFU of SVN-NZ (grown in chick fibroblasts) three weeks apart. One week after the 3rd injection, rabbits were bled and the immune serum saved.

Virus propagation and assay

Virus was propagated and assayed either in mice or in Vero cell cultures. Suckling mice were injected I.P. or I.C. with 0.03 ml of virus dilution and weanling mice were injected I.P. with 0.1 ml. Lethal doses (LD 50) were computed according to the method of Reed and Muench [18]. The methods used for virus plaque assay in Vero cells have been described [19]. To assay viremia levels in virus infected mice, the mice were bled at various time points from the tail vein into special test tubes (brand serum separator, Becton Dickinson) and virus content in the serum was plaque-assayed in Vero cells. To assay virus content in mice brains, 10% brain suspensions in PBS were plaque-assayed in Vero cells.

Immunoassays

Neutralization tests (Plaque reduction) were carried out by mixing equal volumes of a virus dilution which contained 50–100 PFU/0.1 ml with the appropiate serum dilution, incubating

them for 30 min at room temperature and plating them on Vero cell monolayers. After 60 min at 37 °C for adsorption the plates were overlayed with Tragacanth (grade III A-1128 Sigma), and plaques were read 48 h later, stained by neutral-red. HA and HI tests were carried out by conventional methods [4].

Results

Augmentation of neurovirulence of SV

The field strain of SV was passed consecutively by I.C. injection in suckling mice. Every 5th passage the virus preparation was examined for its neurovirulence in weanling mice (I.C. and I.P. injection). At passage 15 the mouse-brain suspension started to cause death in weanling mice injected I.C. but not in those injected I.P. Thereafter, the virus was passed in weanling mouse brains, and at passage 22 it killed mice both I.C. and I.P. injection. Assay of the virus suspension in Vero cell cultures revealed that the first SV passage was composed of large plaques (5 mm in diameter), at P-15 most of the plaques were small (1–2 mm), and at P-22 there was a mixed population of small and large plaques.

The Vero cell assay plates of P-1 and P-22 were used for plaque picking. Three strains were plaque purified three times on Vero cell cultures and were designated SV (large plaque, non virulent), SVN (small plaque, neurovirulent), and SVNI (large plaque, neuroinvasive and neurovirulent).

Validation of identity of the virus strains

To make sure the strains we had chosen were truly Sindbis virus-derived they were tested by NT along with lab-strains of SV, SFV and WNV, against NIH-derived rabbit-anti SV AR339 immune serum and against rabbit anti-SVN-NZ immune serum. They were also tested by HI and cross-protection. The results confirmed the identity of SV, SVN and SVNI as SV-derived strains (Table 1).

However, NT against rabbit anti-SVN-NZ revealed a difference in the NT curve of SVNI, and the titer was a little lower than that of the other strains (Fig. 1).

Infectiousness and growth of the virus strains

Infectivity was assayed by PFU in Vero cells and I.C. and I.P. LD 50 in suckling and weanling mice. These entities were compared with the HA content of the virus suspensions as a measure for the relative number of virus particles. The data in Table 2 demonstrate that all three strains have the same infectiousness towards Vero cell cultures, as measured by PFU/HA ration. There is nearly a 1 : 1 ratio between PFU and I.C. or I.P. LD 50 in suckling mice for all strains. In weanling mice SV is completely non-lethal both by I.C. and I.P. injection, SVN is lethal only by I.C. injection and to the same titer as in suckling mice, and SVNI is lethal by both I.C. and I.P. injection, and the titer is similar to that in suckling mice. The infectiousness to mice of the three strains (i.e., their ability to start a cycle of replication in the mouse which will culminate in death

Table 1. Identification of Sindbis virus strains by plaque reduction and cross protection

Virus strain	50% Plaque reduction		Cross-protection	
	R α Sindbis NIH	RαSVN-NZ	Challenge[a] by SVN	Challenge[a] by SVNI
SVN-NZ	500[b]	12,500[b]	0/5[c]	0/5[c]
SV	500	12,500	0/5	0/5
SVN	500	12,500	0/5	0/5
SVNI	250	5,000	0/5	0/5
SFV	< 20	< 100	5/5	5/5
WNV	< 20	< 100	5/5	5/5
PBS	–	–	5/5	5/5

[a] Challenge by 100 I.C. LD 50 injected to mice 14 days after the I.P. injection
[b] Reciprocal of the serum dilution which caused 50% plaque reduction
[c] Dead out of total injected mice

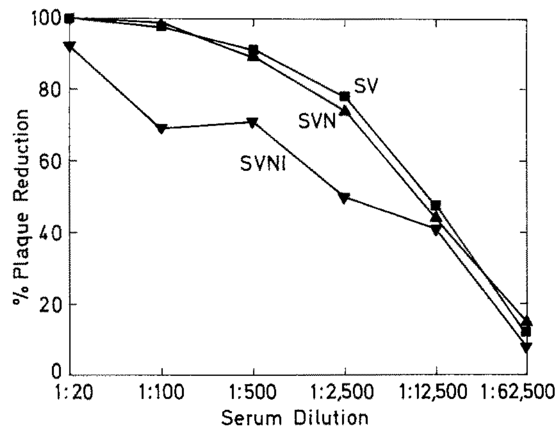

Fig. 1. Neutralization (% plaque reduction) of SV, SVN and SVNI by rabbit-anti-Sindbis-NZ immune serum

Table 2. Comparison of titers of virus stocks of SV, SVN and SVNI assayed in cell culture, suckling mice and weanling mice

Virus strain	PFU/ml	HA titer	Suckling mice		Weanling mice	
			I.C. (LD_{50})	I.P. (LD_{50})	I.C. (LD_{50})	I.P. (LD_{50})
SV	7.0×10^7	1:256	5.28×10^7	3.33×10^7	< 10	< 10
SVN	1.1×10^8	1:256	1.05×10^8	8.56×10^7	5.77×10^7	< 30
SVNI	1.5×10^8	1:512	1.35×10^8	1.05×10^8	5.67×10^7	9.12×10^5 [a]

[a] Includes surviving mice in several dilution groups (see text)

Table 3. Content in plaque forming units of SV, SVN and SVNI in brains of I.C. and I.P. infected suckling and weanling mice

Virus strain	Suckling mice	Weanling mice	
	I.C.[a]	I.C.[a]	I.P.[a]
SV	$3.4 \pm 1.3 \times 10^8$	$2.5 \pm 0.95 \times 10^4$	$2.0 \pm 0.6 \times 10^4$
SVN	$2.0 \pm 0.92 \times 10^9$	$2.2 \pm 0.86 \times 10^7$	< 50
SVNI	$1.82 \pm 0.78 \times 10^9$	$1.6 \pm 0.78 \times 10^6$	$9.37 \pm 1.78 \times 10^5$

Each numerical result is the mean ± S.D. of separate assays of individual brains from 8 infected mice

[a] Brains of suckling mice were harvested on day 2 post infection; brains of weanling mice were harvested on day 5

or a significant serological conversion) is very similar, and after injection I.P. of 10 LD 50 or even less they cause in weanling mice a high-titered viremia (over 10^4 PFU/ml serum, data not shown). The yield of the virus strains in the brain of suckling mice (day 2 post injection) and weanling mice (day 5 post injection) injected I.P. is presented in Table 3. Day 2 (for suckling mice) and 5 (for weanling mice) post injection were chosen because preliminary data showed that maximum yield was achieved at that time. The titer of SV, both in suckling and weanling mouse brains, is lower than that of SVN and SVNI when injected I.C. With SVN there was no virus in weanling mice brains after I.P. injection, even at doses of 10^6 PFU. SVNI injected I.C. or I.P. into weanling mice reached titers of about 10^6 PFU in the brain and caused death of all injected mice. However, there is a qualification to these statements concerning both SVN and SVNI. In SVN, in 3 out of 20 (15%) weanling mice injected I.P. virus could be found in the brain, irrespective of the dilution injected. This was also the pattern of death among mice injected I.P. by SVN. The virus suspension prepared from the brain of these moribund mice had the same qualities as the parent SVN suspension. A similar phenomen was found in SVNI where about 3 weanling mice out of 25 (12%) injected I.P. survived, irrespective of the dose injected. They were completely protected against a challenge of 100 LD 50 of SVNI injected I.C. In SVNI I.P. injected weanling mice all assayed brains contained virus.

Viremia and virus content in the brains of weanling mice injected I.P. by SV, SVN and SVNI

The data shown above pointed towards the possibility that the difference in lethality between the three strains was due to differences in growth capacity and/or invasiveness into the CNS. A detailed study was carried out in weanling mice injected I.P. with 1,000 PFU each of the three strains, and the results are depicted in Fig. 2. The results of the viremia and brain virus levels are based

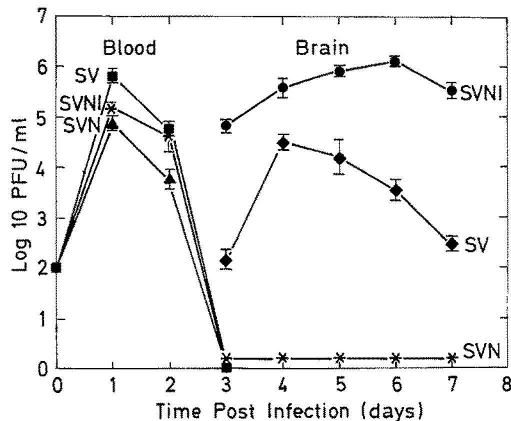

Fig. 2. Time-course of the appearance of SV, SVN and SVNI in blood and brain of ICR weanling mice injected I.P.. Mice were injected with 1,000 PFU each of the 3 virus strains, each data point represents the means of 8 individual assays ± S.D

on the mean of 8 individual assays for each data point. All three strains caused a high viremia evident in less than 24 h post infection, which lasted until 48 h and then subsides; SV had the highest titer, SVNI followed and SVN was last, with an 8 fold difference between SV and SVN. On day 3 no virus could be found in the serum samples from mice inoculated with any of the three strains. The content of virus in the brains was assayed beginning at day 3 post injection, i.e., when no virus was present in the blood. In the case of SVN no clinical signs were visible and no virus was found in the brain until day 8 (except in a few mice, as mentioned above). Both SV and SVNI penetrated into the brain but the difference in titer was dramatic – there were about $10^{4.6}$ PFU of SVNI on day 3 as compared to $10^{2.5}$ PFU of SV per 0.1 g of brain tissue. The peak of the replication was on day 4 for SV ($10^{4.4}$) and day 6 for SVN ($10^{5.0}$). The titer in SVNI persisted with a slight decrease until day 7 when the mice died. In SV the decline in virus titer started on day 5, and by day 8 the clearance was nearly complete. Even though SV viremia was two fold higher than that of SVNI, the amount of virus in the brain on day 3 was over 1,000 fold lower. Also the viremia of SVN was only 2 fold lower than SVNI's, but no virus could be found in the brain. This finding suggested that the viremia does not cause the CNS invasiveness by itself. Thus, SV and SVNI are invasive whereas SVN seems to be non-invasive, even though the viremias they cause are similar.

Invasion of the CNS by SVN through nasal instillation and temporary breach of the blood-brain-barrier (BBB)

It is self evident that infection I.C. of SVN provides an invasion of the brain, and starts a lethal infection with concomitant replication of the virus to a higher yield than that of the other two strains. To circumvent this crude measure, virus may be instilled into the nares thus making it available to the naked tips of the olfactory nerve ends, which are not protected by the BBB. From there the virus may travel into the CNS and start a lethal infection. All weanling mice infected in this way came down with the infection. Virus isolated from brains of moribund mice conformed to the lethality of SVN. Similar results

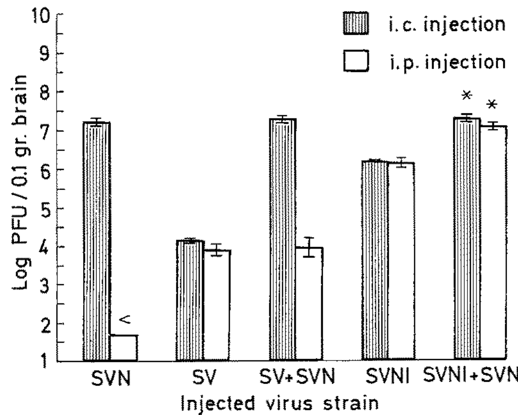

Fig. 3. Invasion into the brain of weanling mice by SVN after injection I.P. together with SVNI. Mice were infected I.C. with 100 PFU and I.P. with 1,000 PFU of each of the 3 virus strains or their combinations. Virus content in the brains on day 5 post injection was assayed in Vero cell cultures. Each numerical result is the mean ± S.D. of separate assays of individual brains from 7 infected mice. * $P < 0.05$ compared to SVNI brain titer

were obtained in weanling mice when SVN (10^4 PFU) was injected I.V. and the mice were then exposed for 2 min to an atmosphere of 27% CO_2 in air, a measure that causes a temporary breach of the BBB [14, 17].

Invasion of the brain of weanling mice by SVN when injected I.P. together with SVNI

Both SV and SVNI invade the CNS after I.P. injection, whereas SVN does not, even though all three cause viremia of similar titer and duration. We wanted to determine whether I.P. injection of SVN together with either of the two other strains would facilitate SVN's co-invasion of the brain. The results of this experiment are presented in Fig. 3. Co-infection of SVN with SVNI by I.P. route resulted in invasion of both SVN and SVNI into the brain. This was shown both by the titer of the yield (10^7 PFU/ml − 10 fold higher than the yield of SVNI injected alone and comparable to the yield of SVN injected alone by I.C. route) and by the mixed population of plaques. Small plaques picked from the yield of brains co-infected with SVN + SVNI caused the same lethality in I.P. and I.C. injected mice as the parent SVN. SV did not facilitate SVN invasion into the brain, while all controls behaved as expected.

Virulence of SVN and SVNI to BALB/c mice

The above results were all obtained in outbred ICR mice. To validate these results in another outbred mouse strain (Webster Swiss) and to relate them to former data, which were mainly obtained in BALB/c mice [9], SV, SVN and SVNI were assayed I.C. and I.P. in Swiss and BALB/c (inbred) weanling mice. The results are presents in Table 4. Mortality for the 3 virus strains in Swiss mice was very similar to that in ICR mice, showing that virulence and invasiveness were the same as in ICR. Virulence (mortality by I.C. injection) in BALB/c mice was comparable to that in the other mouse strains, but neuroinvasiveness of SVNI (mortality after I.P. injection) was much lower. To find out whether the lower invasiveness was linked to differences in extraneural viral

Table 4. Mortality in 3 mouse strains infected I.C. and I.P. with SV and SVNI

Virus	Mice					
	ICR		Swiss		BALB/c	
	Titer LD 50/ml[a]					
	I.C	I.P.	I.C.	I.P.	I.C.	I.P.
SV	< 10	< 30	< 10	< 30	< 10	< 30
SVN	5.8×10^7	< 30	5.6×10^7	< 30	5.3×10^7	< 30
SVNI	5.6×10^7	9.1×10^5	5.9×10^7	5.3×10^6	4.8×10^7	1.0×10^3

[a] Titer (PFU/ml) of infecting stocks SV: 7.0×10^7; SVN: 1.1×10^8; and SVNI: 1.5×10^8

replication in BALB/c mice, viremia levels were compared between Swiss and BALB/c mice injected I.P. The results are presented in Table 5. Viremia levels in Swiss mice are 10 fold higher than in BALB/c mice, and its duration is longer; all the I.P. injected Swiss mice succumbed at day 6–7, whereas in BALB/c mice only 3 were dead out of 6 injected I.P. with 5×10^4 PFU and none among those injected with 5×10^3 PFU, even though the viremia following both inoculae was similar.

Stability of the characters of virulence and neuroinvasiveness

Both derived strains, SVN and SVNI, were assayed for their virulence and neuroinvasiveness (PFU/ICLD 50/IPLD 50) following three consecutive passages each in Vero cells, C6/36 insect cells and ICR weanling mice injected I.C. No change in virulence and neuroinvasiveness was noticed (data not shown).

Table 5. Viremia on day 1 and 2 post infection in Swiss and BALB/c mice injected I.P. with SVNI

Mice	Inoculum					
	5×10^3 PFU/mouse			5×10^4 PFU/mouse		
	Day 1[a]	Day 2[a]	Mortality[b]	Day 1[a]	Day 2[a]	Mortality[b]
Swiss	$4.0 \pm 2.1 \times 10^4$	$5.5 \pm 3.5 \times 10^2$	6/6	$2.8 \pm 2.0 \times 10^4$	$1.5 \pm 1.3 \times 10^2$	6/6
BALB/c	$3.2 \pm 1.5 \times 10^3$	< 50	0/6	$3.5 \pm 1.7 \times 10^3$	< 50	3/6

[a] PFU/ml mouse serum
[b] Dead/injected

Discussion

Extensive studies of Sindbis virus have been carried out with strain AR339 or its derivatives. This strain was isolated in Egypt in the early 50's [21]. Our strain of SV was isolated from mosquitoes in southern Israel in the early 80's, and there is great similarity between the two isolates in their antigenicity and virulence in suckling and weanling mice [6, 11].

Of our two virus strains, derived by serial passage in suckling and weanling mouse brains, SVN seems similar, according to published data [8, 10], to NSV on account of its neurovirulence and almost complete inability to invade the CNS of weanling mice after peripheral injection. The second strain, SVNI, seems to be, to the best of our knowledge, a new Sindbis virus strain with previously undescribed properties. All three strains replicate well in the outbred ICR mouse after peripheral injection and cause a high viremia. They differ, however, in their subsequent interaction with the CNS. In this context it is useful to consider the capacity of a virus to establish a lethal infection within the CNS as the property of neurovirulence and the ability to penetrate the CNS after inoculation and growth at a peripheral site as the property of neuroinvasiveness [5, 11, 12, 113]. Thus in weanling mice, SV is neuroinvasive but non-neurovirulent, SVNI is neurovirulent and neuroinvasive and SVN is neurovirulent but non-neuroinvasive, even though a high titer viremia is maintained.

The difference in neurovirulence between SV-AR339 and NSV stems from the ability of NSV to infect many more neurons than SV does in the CNS of BALB/c mice, thus causing a much more severe pathology which culminates in death [10]. This is probably also the reason for the difference in virulence in our system although this has yet to be verified. It should be stressed that the neurovirulence of SVN is very similar to that of SVNI, where 1–3 PFU consitute 1 LD 50, following viral CNS replication and acute encephalitis.

The way neuroinvasiveness is expressed depends on the virus – it may be done by neural spread as in rabies, herpes or reovirus type 3 [22] or by haematogenous spread (arboviruses, enteroviruses, measels and mumps) starting with viremia which is followed by CNS invasion [5, 22]. The mechanism of invasion is not always known.

We have shown that both SV and SVNI are present in the CNS of weanling mice, already on the 3rd day following I.P. injection, whereas SVN (except in about 15% of I.P. injected mice) does not invade the CNS, while all three cause a high level viremia of similar duration. In the case of SVN the presence of large numbers of virions in the blood does not facilitate penetration into the CNS through the BBB. It was shown [16] that in the case of hamsters, infected I.P. with SLE, virus penetrated the CNS via the olfactory nerve-ends located in the buccal cavity. As SVN can penetrate into the CNS following intranasal instillation, it is evident that in spite of the substantial viremia which arises after I.P. injection it cannot cross the olfactory pathway.

Our assumption that the penetration into the CNS of SV and SVNI take

effect through a physical breach of the BBB was corroborated for SVNI only by circumstantial evidence. Double infection I.P. by SVN and SVNI resulted in penetration of both virus strains into the CNS whereas SVN itself cannot do that. In the case of double infection, the viremia of both virus strains coincide and SVN particles follow SVNI into the CNS. In the case of SV, which is also neuroinvasive, double infection I.P. with SVN does not facilitate SVN penetration into the CNS, even though the same conditions prevail as in the case of SVNI. It is possible that the mechanism of CNS penetration by SV differs from that of SVNI.

The penetration of the CNS by SVN when coinjected I.P. with SVNI suggests an analogy with SVN's penetration into the CNS following a temporary breach of the BBB of the mouse, achieved by breathing a mixture of 27% CO_2 in air. It is also similar to the penetration into the CNS of WN_{25}, a non-neuroinvasive mutant strain of the neuroinvasive West Nile virus, following BBB-modulation by various means [2, 14]. How this breach of the BBB takes place in concord with the extraneural replication of SVNI in the mouse is unknown. It could be mediated by some lymphokine arising as a result of the virus interaction with mouse tissue, similar to that produced during Dengue 2 infection in mice [3]. SVN, presumably, has a much lower capacity for triggering this reaction. This may be the reason for its partial non-neuroinvasiveness, i.e., 15% of the outbred weanling mice injected I.P. with SVN, had virus in the CNS, whereas all the rest were completely negative. The survival of 12% of weanling mice injected I.P. with SVNI is probably due to the fact that the outcome of a viral infection depends on the balance between viral replication and host immunity. As the hosts in this case are outbred mice, this balance may differ individually.

The measures of neuroinvasiveness of SV, SVN and SVNI were similar when assayed in two different outbred mouse populations. It may be concluded that these measures are a true expression of the 3 virus phenotypes. A similar measure of neurovirulence was displayed when the 3 viruses were assayed in inbred weanling BALB/c mice (measured by ICLD 50/PFU), but neuroinvasiveness of SVNI (IPLD 50/PFU) was much lower. Also the level of viremia was lower and of shorter duration than in outbred mice, but it was similar in BALB/c mice infected I.P. with varying virus doses. This viremia, however, was presumably sufficient to seed CNS infection if virion availability were the limiting factor; no virus could be found in the CNS of most of the I.P. infected mice. Thus the lower neuroinvasiveness of SVNI in weanling BALB/c mice may be linked to the lesser viremia or to resistance at the level of the ability to breach the BBB.

These three SV strains may be useful in extending the data concerning the molecular determinants of neurovirulence [15] and in providing first-hand knowledge about the sequence(s) which control the neuroinvasiveness in SVNI. Our laboratory is now engaged in sequencing our SV strain and its comparison with other, known strains of SV [20].

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References

1. Akov Y (1976) Some properties of togavirus haemagglutinin studied with the aid of kaolin-adsorbed virus. Arch Virol 51: 99–106
2. Akov Y, Halevy M, Lustig S (1987) Neuroinvasiveness of west nile virus (WNV) strains and the use of modulators of the blood brain barrier (BBB). In: Proceedings of the 7th International Congress of Virology, Edmonton, Alberta, Canada, p 74
3. Chaturvedi UC, Khanna M, Dhawan R, Mathur A (1990) Dengue virus-induced cytokines increase capillary permeability. In: Proceedings of the 8th International Congress of Virology, Berlin, p 470
4. Clarke DH, Casals J (1958) Techniques for haemagglutination-inhibition with arthropod borne viruses. Am J Trop Med 7: 561–573
5. Gonzalez-Scarano F, Tyler KL (1987) Molecular pathogenesis of neurotropic viral infections. Ann Neurol 22: 565–574
6. Griffin DE (1986) Alphavirus pathogenesis and immunity. In: Schlesinger S, Schlesinger MJ (eds) The Togaviridae and Flaviviridae. Plenum, New York, pp 209–249
7. Griffin DE (1989) Molecular pathogenesis of sindbis virus encephalitis in experimental animals. Adv Virus Res 36: 255–257
8. Griffin DE, Johnson RT (1977) Role of the immune response in recovery from sindbis virus encephalitis in mice. J Immunol 118: 1070–1075
9. Griffin DE, Hahn SC, Jackson AC, Lustig S, Strauss EG, Strauss JH (1989) The basis of sindbis virus Neurovirulence. In: Compans R, Helenius A, Oldstone M (eds) Cell biology of virus entry, replication, and pathogenesis. AR Liss, New York, pp 387–396
10. Jackson AC, Moench TR, Trepp BD, Griffin DE (1988) Basis of neurovirulence in sindbis virus encephalomyelitis of mice. Lab Invest 58: 503–509
11. Johnson RT (1965) Virus invasion of the central nervous system: a study of sindbis virus infection in the mouse using fluorescent antibody. Am J Pathol 46: 929–943
12. Johnson RT (1982) Viral infection of the nervous system. Raven Press, New York
13. Johnson RT, Mims CA (1968) Pathogenesis of viral infections of the nervous system. New Engl J Med 278: 84–92
14. Kobiler D, Lustig S, Gozes Y, Ben-Nathan D, Akov Y (1989) Sodium dodecylsulphate induces a breach in the blood brain barrier and enables a west nile virus variant to penetrate into mouse brain. Brain Res 496: 314–316
15. Lustig S, Jackson AC, Hahn CG, Griffin DE, Strauss EG, Strauss JH (1988) Molecular basis of sindbis virus neurovirulence in mice. J Virol 62: 2329–2336
16. Monath TP, Cropp CB, Harrison AK (1983) Mode of entry of a neurotropic arbovirus into the central nervous system. Lab Invest 48: 399–410
17. Rapoport ST (1976) Blood-brain barrier in physiology and medicine. Raven Press, New York
18. Reed LJ, Muench H (1938) A simple method of estimating fifty percent end points. Am J Hyg 27: 493–497
19. Shahar A, Lustig S, Akov Y, David Y, Schneider P, Levin R (1990) Different pathogenicity of encephalitic togaviruses in organotypic cultures of spinal cord slices. J Neurosci Res 25: 345–352
20. Strauss EG, Rice CM, Strauss JH (1984) Complete nucleotide sequence of the genomic RNA of sindbis virus. Virology 133: 92–110
21. Taylor RM, Hurlbut HS, Work TH, Kingsbury RH, Frothingham TE (1955) Sindbis

virus: a newly recognized arthropod-transmitted virus. Am J Trop Med Hyg 4: 844–846
22. Tyler KL, McPhee DA, Fields BN (1986) Distinct pathways of viral spread in the host determined by reovirus S1 gene segment. Science 233: 770–774

Authors' address: Dr. S. Lustig, Department of Virology, Israel Institute for Biological Research, P. O. Box 19, Ness Ziona 70450, Israel.

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