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Defective Interfering Virus Particles Modulate Virulence

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To determine whether defective interfering (DI) particles modulate virulence by initiating a cyclic pattern of virus growth in vivo, adult mice were infected with vesicular stomatitis virus (VSV), both with and without DI particles. A total of 184 mice divided into groups were inoculated intranasally. A majority of mice inoculated only with standard VSV developed paralysis, most of them between days 7 and 9. The addition of DI particles altered the development of paralysis in several ways. When there was significant protection, a few still became paralyzed on days 7 and 9. When overall mortality was unaffected or even slightly increased, the majority of mice became paralyzed between days 7 and 9 as well. Protection could not be predicted based on a single ratio of standard VSV to DI particles or on the absolute amount of DI particles inoculated. Infectious virus recovered from mouse brains at the time of paralysis and incipient death showed considerable variation, although the titer in a majority of the animals was between 10^5 and 10^7 PFU/ml. When the brains of these paralyzed mice were examined for hybridizable VSV RNA, the detection of standard VSV RNA correlated well with infectivity. The amount of DI RNA in the coinfected mice was more variable and independent of the amount of 40S RNA, although DI RNA was usually found when standard RNA was present. Survivors examined between days 14 and 21 did not contain infectious virus or any detectable viral RNA in their brains. Because these results were consistent with the hypothesis of viral cycling in vivo, rather than a gradual accumulation of total infectious virus, mice were coinfected with 10^8 PFU of standard VSV and 10^5 PFU equivalents of DI particles and sacrificed daily thereafter, irrespective of whether they developed paralysis. Infectivity measurements indicated a reproducible cycling pattern of VSV in the mouse brains with a periodicity of about 5 days. This cycling and the detection of DI RNA in brains several days after intranasal inoculation suggest that there is a dynamic continuous interaction between standard VSV and its DI particle beyond the initial site of replication as the virus population spreads into the host animal. Such cycling of virus production before the full development of specific immune responses from the host may have important implications for viral diagnostics and disease transmission.

Defective interfering (DI) particles of animal viruses have been characterized for almost all groups of animal viruses (7, 10, 15). Despite considerable work on their molecular biology, their role in viral pathogenesis remains unclear. Because of their ability to inhibit the growth of their standard helper virus in cell culture, it has been postulated that DI particles may be natural modulators of viral infections (9). Lethal infections of suckling mice with vesicular stomatitis virus (VSV) show an ameliorating effect by DI particles only when their concentration is increased to 5×10^{10} PFU equivalents or to a dose greater than 1,000-fold that of standard virus (4–6, 16). At lower doses, DI particles prolong the disease symptoms, which eventually lead to death (6). These results confirm the finding that young mice are highly susceptible to VSV infection (19) and suggest that their susceptibility may be because they are refractory to the protection afforded by DI particles.

Lethal infection of adult mice with VSV requires more virus in the inoculum (8). However, coinfection with DI particles and standard virus at a ratio of 0.001:1 shows significant protection (3). These results are unexpected from one-step viral growth studies in cell cultures where this ratio of DI and standard virus does not prevent the replication of standard virus to any significant degree (11, 24). Moreover, in the same studies with adult mice a 1:1 ratio of DI particles to standard virus fails to protect mice, whereas in cell cultures replication of standard virus is completely aborted (3, 11, 24). From these studies with adult mice it is clear that the outcome of infections in vivo cannot be predicted from one-step growth studies of VSV mixtures in cell cultures. Also, these infections in vivo do not appear to follow a pattern of continued increase in virus titers over time with the timing of paralysis and death dependent on the effective initial inoculum of standard virus.

Rather, the results with adult mice are suggestive of a cycling pattern of virus production (3), more like sequential undiluted passaging of viruses in cell cultures (14, 23). Within five of these undiluted passages the standard virus titers decrease and then increase, forming a cyclic pattern of production with the synthesis of DI particles following slightly behind the synthesis of standard virus. This cycling continues indefinitely in cell cultures as long as the cells are not limiting. Therefore, the interaction of DI particles with standard virus in the cell culture resembles the classical predator-prey relationship. If VSV is cloned and purified in such a way so as to be rid of all DI particles, then continuous undiluted passaging does not result in a cyclic production of standard virus until seven successive passages have taken place (unpublished data).

Therefore, if cycling occurs in vivo it is possible that infections of mice could be initiated with different concentrations or populations of VSV and end up with the same

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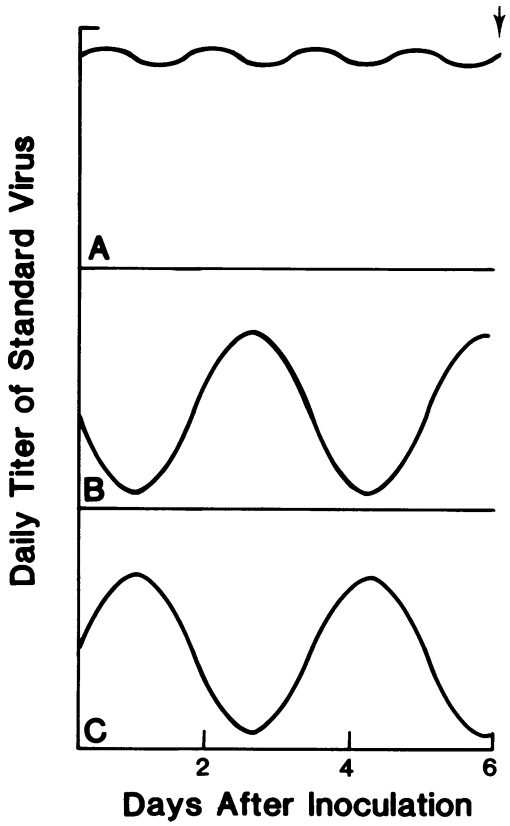

FIG. 1. Hypothetical outcomes of viral infections with and without cycling due to DI particles. Panels: A, infection with standard virus alone; B, infection with equal amounts of DI and standard virus; C, infection with DI and standard virus at a ratio of 0.01:1. The arrow illustrates the concentration of standard virus that can be obtained at a critical later time. This schematic drawing was adapted from previously published data (14).

amount of standard virus after several days of growth (Fig. 1, lines A and B). Also, the same amount of standard virus in the inoculum together with different concentrations of DI particles could lead to opposite cycling patterns with very different concentrations of virus after several days of growth (lines B and C). If the critical period in the infected animals were 6 days after infection, as indicated by the arrow, then one might predict that animals showing patterns A and B would have high mortality rates, and animals showing pattern C would have lowered mortality. Such explanations are consistent with the findings reported by Cave et al. (3).

Another prediction of in vivo cycling is that DI RNA in mouse brains would not necessarily correlate with the presence of infectious standard virus or the detection of full-sized standard RNA; in fact, because of overlapping cyclic patterns, the amounts of each of the two RNA molecules could exist at many relative concentrations. Also, the degree of protection offered by DI particles cannot be dependent on the concentration of DI particles alone or on any single ratio of DI to standard virus because the periodicity and the amplitude of the cycling may be altered, depending on the amount of infectious standard virus. Since these patterns are unknown in the animal at present, the critical concentrations of virus reached later in the animal are unpredictable. Lastly, if enough virus can be found in the animal before the development of disease symptoms, cycling of the infectivity and DI particle synthesis may be directly demonstrable.

The mouse model for studying VSV encephalitis is well defined (8, 12, 13, 19–21, 25). Because these infections result in death within 7 to 10 days, the incubation period can be studied before complications are introduced by significant specific host immune responses. To overcome the considerable variation in disease expression from animal to animal, we have developed a highly sensitive and reproducible nucleic acid hybridization technique to detect VSV and DI particle RNAs in individual mouse brains (3). This was used in conjunction with plaque assays in order to measure the degree of virus replication and the relative concentrations of standard infectious VSV and DI particles. Despite the inherent technical difficulties in such animal studies, the results can be analyzed in terms of cyclic synthesis in vivo.

MATERIALS AND METHODS

Virus preparations. The growth, purification, and quantitative assay of standard VSV of the Indiana serotype, San Juan strain, and its defective interfering particle, DI-T, were as previously reported (3, 11, 24). Stock preparations of standard VSV at 10^{10} PFU/ml and DI-T particles at 10^{10} PFU/ml equivalents were stored at −70°C (11).

cDNA probe. A cDNA probe of approximately 800 base pairs was prepared from DI-T RNA by reverse transcription and insertion in a pBR322 vector as previously described (2, 3). This probe is specific for VSV genome-sized 40S RNA, L mRNA, and DI-T RNA (3). Plasmid pBR322 containing the cDNA probe was nick translated (18) in the presence of [^{32}P]dCTP (New England Nuclear Corp., Boston, Mass.) (specific activity 3,200 Ci/mmol) to a specific activity of greater than or equal to 10^8 cpm/μg of DNA.

Infection of mice. CD1 outbred mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were obtained as retired breeders aged 9 to 10 months. They were caged in groups of four to six animals and housed in a laminar flow cabinet. They were fed standard laboratory mouse chow and given water ad libitum. A 12-h alternating light or dark cycle was used. These mice, along with other CD1 mice obtained from the same distributor over a number of years, were randomly tested for preformed, neutralizing antibody against VSV. None has ever been detected. After 1 week of quarantine, groups of six to eight mice were inoculated intranasally with 20 μl during the hyperventilation phase of recovery from ether anesthesia. The inoculum contained phosphate-buffered saline with either purified standard VSV alone or standard VSV administered with DI particles. Controls were also inoculated with phosphate-buffered saline alone or with DI particles alone. Animals were checked daily for up to 21 days for the onset of hind limb paralysis, which was usually associated with death within 24 h. Mice were sacrificed when paralysis was obvious. Those that died unexpectedly were discarded as the degree of brain autolysis was unknown. The brain, with 0.5 to 1.0 cm of the proximal spinal cord including particularly the olfactory bulbs, was removed, cut in half sagitally, and stored at −70°C.

Infectivity assay. Each brain was weighed, and one-half of each was made 10% (wt/vol) in phosphate-buffered saline, homogenized, and centrifuged at 2,500 rpm for 10 min. The supernatant was assayed by plaque formation on Chinese hamster ovary cells (24).

RNA assay. For [^{32}P]cDNA:RNA (Northern) hybridization analysis, the other half of each brain was homogenized and extracted with guanidine thiocyanate, and RNA was

TABLE 1. Mortality of mice with respect to time and inocula

Inoculum (PFU)			No. of deaths on day of paralysis:										Total dead	% mortality
Standard VSV	DI particles	No. of mice	6	7	8	9	10	11	12	13	14	15	18		
10^6		25	0	6	5	1	0	0	0	0	0	0	0	12	48
10^7		25	0	9	2	3	1	1	0	0	0	0	0	16	64
10^8		30	1	8	9	1	1	0	0	0	0	0	0	20	67
10^6	10^5	10	0	1	0	1	0	1	0	0	0	0	0	3	30
10^6	10^6	10	0	3	0	1	0	0	0	0	0	0	0	4	40
10^6	10^7	10	0	1	0	0	0	0	0	0	0	0	0	1	10
10^6	10^8	10	0	0	0	0	1	0	0	0	0	0	0	1	10
10^7	10^5	7	0	5	0	0	0	0	0	0	0	0	0	5	71
10^7	10^6	7	0	1	1	0	0	0	0	0	0	0	0	2	29
10^7	10^7	7	0	1	0	0	1	0	0	0	0	1	1	4	57
10^7	10^8	7	0	1	0	0	0	0	1	0	0	0	0	2	29
10^8	10^5	8	0	1	2	1	0	0	0	0	0	0	0	4	50
10^8	10^6	8	0	0	2	1	0	0	0	0	0	1	0	4	50
10^8	10^7	8	0	1	2	3	0	1	0	0	0	1	0	7	88
10^8	10^8	8	1	3	0	2	0	0	0	0	0	1	0	7	88

centrifuged through a cesium chloride step gradient as previously described (3). After washing, ethanol precipitations, and quantitation at A_{260}, the pelleted RNA was stored at −70°C in H_2O at 5 μg/μl. Although the total amount of RNA extracted was highly variable relative to the weight of the brain, 28 μg of RNA from each mouse was denatured in 20 mM 3-(*N*-morpholine)propanesulfonic acid (MOPS) buffer (Sigma Chemical Co., St. Louis, Mo.) (pH 7.3) containing 50% deionized formamide, 5 mM sodium acetate, 1 mM EDTA, and 6% formaldehyde for 10 min at 60°C and then rapidly cooled on ice. The RNA was electrophoresed in 1% agarose gels containing 6% formaldehyde at 4°C for 20 h at 4 V/cm with a running buffer containing 5 mM sodium acetate, 1 mM EDTA, and 20 mM MOPS (pH 7.3). The transfer of RNA by passive blotting onto nitrocellulose paper (BA85; Schleicher and Schuell, Inc., Washington, D.C.) and hybridization with [^{32}P]cDNA were performed as previously described (3).

RESULTS

Effects of varying standard virus and DI particles on the time of paralysis and overall mortality. To determine whether there was a pattern of protection related to the absolute amount of DI particles or to a particular ratio of DI to standard virus, the concentration of both viral preparations was varied over a 3- to 4-log range in the initial intranasal inoculation. The concentrations (10^6 to 10^8 PFU) of standard VSV chosen had been previously found to result in a mortality of 50% or more in adult mice within an 8-day period (8); lower concentrations reduce mortality and delay the onset of disease. Among the mice infected with standard VSV alone, paralysis occurred between days 6 and 11, mostly between days 7 and 9 (Table 1). In each of the groups, seven mice were female and the rest were male; when their mortalities were compared, there was no significant difference.

Mice coinfected with DI particles and standard VSV showed a wide range of mortality, 10 to 88%, with paralysis developing between days 6 and 11 (Table 1). A few groups showed delayed paralysis in one or two mice, but this delay did not correlate with overall reduced mortality. The degree of protection also failed to correlate with an absolute amount of DI particles or with a particular ratio of standard virus to DI particles in the inoculum. Control mice inoculated with phosphate-buffered saline or 10^8 PFU equivalents of DI particles did not show disease symptoms throughout the observation period.

Correlation of paralysis with infectivity and the detection of genome-sized RNA. To determine whether paralysis and death correlated with the amount of infectious VSV, the brains from the mice infected with standard virus alone were assayed for infectivity by plaque formation and for genome-sized RNA by Northern hybridization.

The results with three groups of mice inoculated only with standard VSV are shown in Table 2. Virus titers in mouse brains at the time of paralysis were generally between 10^5 and 10^7 PFU/ml. Correlations with 40S genomic RNA detected by Northern hybridizations held up for 18 of 24 brains (Table 2). No 40S RNA was detectable below 5×10^3 PFU. The surviving mice were sacrificed on day 14, and their brains were assayed similarly. Neither infectious VSV nor viral RNA was detectable. Variations in the amount of RNA extracted from each mouse were greater than the variations in the weight of brain tissue (mean weight, 515 mg), suggesting either loss during RNA extractions or inherent variation due to viral cytopathic effects. Nevertheless, on repeat experiments the data were in general quite reproducible. Group 3 yielded the best correlations between PFU and RNA, probably because of the experience of the investigator in all the procedures involving RNA.

DI RNA was not detected in any of the mice infected with standard VSV alone. This could be due to the fact that within the short period of the experiment, purified standard virus failed to generate any DI particles. Similar failure to generate DI particles was found by Jones and Holland (12) in young mice and with the continuous passaging of cloned standard VSV in cell cultures where DI particles were not detected for seven undiluted passages (unpublished data). However, it may be equally possible that the probe, which detects only a portion of the L gene, missed the DI particles with nonhybridizable sequences (22).

DI RNA in brains of mice coinfected with standard VSV and DI particles. To determine whether detection of DI RNA in mouse brains correlated with some protection, the cDNA

TABLE 2. Detection of viral RNA in brains of mice infected with standard VSV

Group	Inoculum (PFU)	Mouse no.	Total RNA (μg/g of brain)	Infectivity (PFU/ml of brain)	Hybridizable 40S VSV RNA	Day of sacrifice
1	10^6	1	563	3.7×10^6	+	7
		2	551	2.4×10^6	+	8
	10^7	1	716	5.1×10^5	+	7
		3	249	3.2×10^5	+	9
		4	215	3.0×10^3	−	11
	10^8	1	521	1.6×10^7	−	7
		2	311	2.3×10^5	+	7
		3	215	2.78×10^6	−	8
2	10^6	1	287	5.2×10^6	−	7
		2	455	2.8×10^5	−	8
		3	932	4.2×10^5	−	8
		4	657	$\leq 10^3$	−	14[a]
	10^7	1	270	8.5×10^2	−	7
		2	763	6.2×10^5	+	7
		3	390	5.6×10^3	+	7
		4	825	6.2×10^5	+	8
	10^8	1	732	3.7×10^6	+	7
		2	734	2.1×10^7	+	8
		3	340	4.6×10^5	−	8
		6	672	$\leq 10^3$	−	14[a]
3	10^6	1	820	6.0×10^5	+	7
		2	641	$<10^3$	−	9
	10^7	1	634	8.0×10^5	+	7
		2	129	1.1×10^6	+	7
		3	653	3.1×10^5	+	9
		4	575	2.3×10^5	+	9
	10^8	1	483	6.3×10^6	+	7
		2	657	9.4×10^5	+	7
		3	826	1.7×10^6	+	8
		4	645	6.1×10^6	+	8

[a] Without paralysis and sacrificed.

probe was used to hybridize RNA from the brains of mice coinfected with standard virus and DI particles. Representative results from the coinfected mice shown in Table 1 are presented in Table 3. There was a wide range of infectivity results, indicating considerable individual variability in these coinfected mice, especially in those developing paralysis on day 10 or later. Nevertheless, the detection of DI RNA correlated well with the presence of more than 5×10^4 PFU/ml and genome-sized VSV RNA. Only one mouse demonstrated detectable DI RNA without genome-sized RNA, but then the infectivity was at 6×10^3 PFU/ml (Table 3). Depsite encouraging preliminary results suggesting that detection of DI RNA in paralyzed mice correlates with overall reduced mortality (3), the data here only partially support that contention. However, since these mice were sacrificed only after developing paralysis, it would be expected that the results would be biased in favor of detecting infectious standard virus. It can be concluded that DI RNA was detected only when the inoculum contained DI particles. Again, given the limitations of the hybridization probe, no new DI particles of another size were generated in these coinfected mice. The mice that failed to develop paralysis were kept for 21 days, after which they were sacrificed and their brains were assayed for infectivity and hybridizable VSV RNA. Neither could be detected (data not shown).

Quantitation of relative amounts of 40S and DI RNA. According to the hypothesis presented above, DI RNA could be detected alone or together with standard virus RNA because of competition between the synthesis of the two RNAs. To see whether the two RNA species covaried or whether one increased at the expense of the other, Northern hybridization results were examined more closely. When selected mice inoculated with either 10^6 or 10^8 PFU of standard VSV and various dilutions of DI particles were compared, occasionally no viral RNA was found at all; but in others, VSV RNA and DI RNA were detected. The amounts of DI RNA and standard RNA appeared to vary, supporting their production in an overlapping cyclic pattern (Fig. 2, lanes 9 through 12). These observations suggest that there is continuous interaction between standard and DI VSVs beyond the initial site of replication and that such cycling was established by the DI particles contained in the initial inoculum. The failure to detect any viral RNA in some mice

TABLE 3. Detection of viral and DI RNAs in brains of mice coinfected with VSV and DI-T

Inoculum PFU		Mouse no.	Total RNA (μg/g of brain)	Infectivity (PFU/ml of brain)	Hybridizable RNA		Day of sacrifice
Standard VSV	DI particles				VSV	DI particles	
10^6	10^5	2	968	2.5×10^5	+	−	9
		3	791	0	−	−	11
10^6	10^6	2	818	6.2×10^4	+	+	6
		4	904	2×10^3	−	−	8
10^6	10^7	1	838	6×10^3	−	+	7
10^6	10^8	1	ND	ND	ND	ND	10
10^7	10^5	2	721	4.0×10^6	+	+	7
		3	716	4.5×10^5	−	−	7
		4	755	2.2×10^5	−	−	7
		5	580	3.4×10^5	+	+	7
10^7	10^6	1	655	2.5×10^6	+	+	7
		2	896	9×10^6	+	+	8
10^7	10^7	2	679	7.8×10^5	+	−	10
		3	706	0	−	−	15
		4	934	0	−	−	18
10^7	10^8	1	840	1.5×10^5	0	0	7
10^8	10^5	1	769	3.05×10^5	+	+	7
		2	560	ND	+	+	8
		3	375	2.6×10^5	+	−	8
10^8	10^6	1	419	2.6×10^6	+	+	8
		4	1,028	0	−	−	15
10^8	10^7	1	1,004	2.7×10^5	+	+	7
		3	441	1.0×10^5	−	−	8
		5	663	7.5×10^2	−	−	9
10^8	10^8	1	758	1.3×10^5	+	−	6
		2	720	1.5×10^6	+	−	7
		3	689	ND	+	−	7
		4	473	6×10^4	+	−	7
		5	878	1.25×10^6	−	−	7
		6	1,021	1×10^5	−	−	7
		7	964	0	ND	ND	15

[a] ND, Not done.

may be due to technical problems or to the fact that when the mice were sacrificed, their degree of paralysis was not always the same, with some of them nearly moribund and others only limping slightly.

Cycling of infectious VSV in mouse brains. Because the findings so far were consistent with the hypothesis of cycling between standard virus and DI particles in animals, an attempt was made to examine the latent period immediately after virus inoculation and determine whether virus production and cycling could be measured in a sequential way. Such sampling has been avoided by us and others because it was felt that there would not be enough virus produced early during infection. Mice were inoculated with 10^8 PFU of standard VSV and 10^5 PFU equivalents of DI particles, a concentration which permitted ready detection of both standard VSV and DI particles at the time of paralysis. Two mice were then sacrificed on each day for the following 11 days, and their brains were processed for infectivity assays. This experiment was repeated with good reproducibility on two batches of mice.

Figure 3 shows one of the assay results, with a line drawn to indicate the average titer of VSV found in the two mouse brains. Not only was infectious virus readily detected from day 1, but subsequent cycling of infectivity was found. The arrows indicate plaque assays where the presence of DI particles was suspected because in the various dilutions, plaque counts deviated from linearity. To obtain a better estimate for the presence of DI particles, each of the mouse brains was extracted for RNA and blotted. Unfortunately, the yield of virus-specific RNA from days 0 to 5 was so low that positive results were not obtained after Northern hybridizations. However, with mice similarly infected with 10^8 PFU of standard VSV and 10^6 DI particle equivalents on day 5 one mouse showed a very positive band at the position expected for size of RNA of input DI particles (Wu and Huang, unpublished data). Titers for the first 2 days were

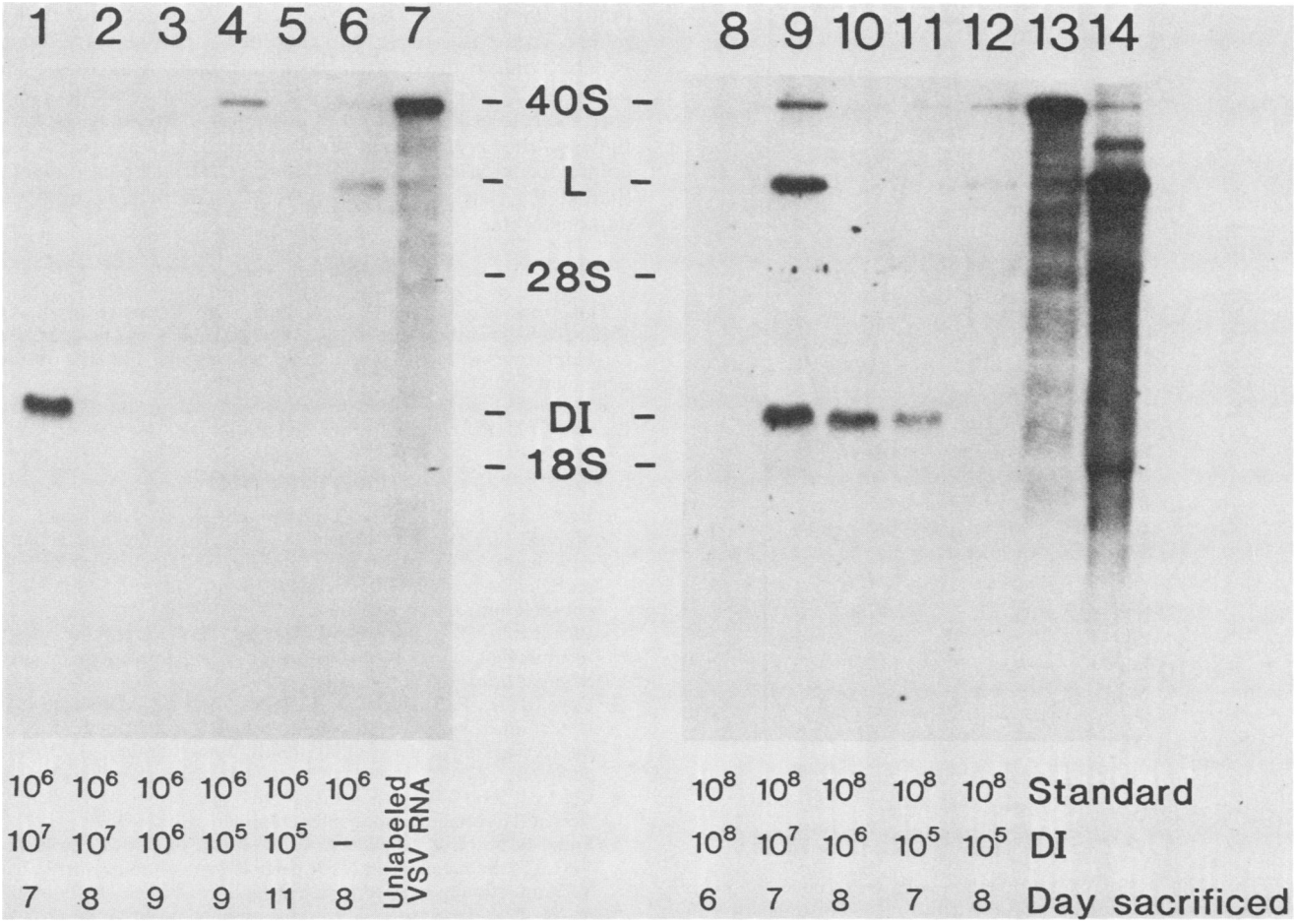

FIG. 2. Gel electrophoresis of RNA from individual mouse brains detected by Northern hybridization. RNA extracted from brains of mice infected with various combinations of standard VSV and its DI-T particles were separated on 1% agarose gels, blotted onto nitrocellulose paper, and hybridized with clone 6 [^{32}P]DNA (16). Abbreviations: 40S, genome-sized RNA; L, L mRNA; DI, DI-T RNA; 28S and 18S, rRNA markers. Lane 13 was identically loaded as lane 7 with 250 ng of genomic RNA, and lane 14 contains ^{32}P-labeled VSV RNAs from infected cells.

probably underestimates, because it has been found that some initial viral replication occurs in the upper respiratory tract (25).

DISCUSSION

These studies indicate that the time between exposure to VSV and development of disease symptoms was a period when measurable active viral replication occurred. The progression of the viral spread appeared to continue to involve DI particles that were present in the inoculum. The dynamics may have led to an oscillatory synthesis of infectious virus, sometimes resulting in protection of mice from the lethal effects of VSV. A predictive relation between the cyclic patterns and protection from or enhancement of disease symptoms by DI particles will require multiple serial studies on both infectivity and viral RNA synthesis in mouse brains after inoculation with different viral mixtures.

The apparent critical period for the disease in this system was around 7 to 9 days. The mice which did not develop paralysis during this period had a much greater chance of survival. Those that survived lost residual detectable infectivity and viral RNA. This critical period coincides with the time when immune defenses of adult mice are thought to reach full activity against new antigens. Therefore, the cycling pattern between DI particles and standard VSV most likely occurred before specific host immune responses were fully developed. The outcome of the disease may depend on the ability of the host to overcome virus spread and prevent destruction of brain tissue. If the virus population at the time of mounting host defenses is such that there are large amounts of DI particles and little infectious VSV, the host may have a better chance of resisting the infection.

In mouse brains, the cycling of infectivity appeared to be remarkably similar to passage in cell cultures (14, 23) (Fig. 3). One complete oscillation or period takes four to five 24-h passages. Also, during such continuous passaging in vitro, infected cells show a variety of responses. Whether cells are viable or whether they contain a large amount of viral antigens and nucleic acids depends on the total amount inoculated and the relative ratios of standard VSV and DI particles (14). If DI particles are not in the initial inoculum, the virus titer remains high, most of the cells are killed, and DI particles are not detectable for seven undiluted passages (unpublished data).

If the cycling hypothesis holds true in vivo, there are several important implications. For example, in viral diagnosis, if sampling of individuals occurs when infectious virus is cycling, then variations in isolating infectious virus are to be expected. Because of this, diagnosis would require, in addition to sensitive infectivity assays, assays for detecting

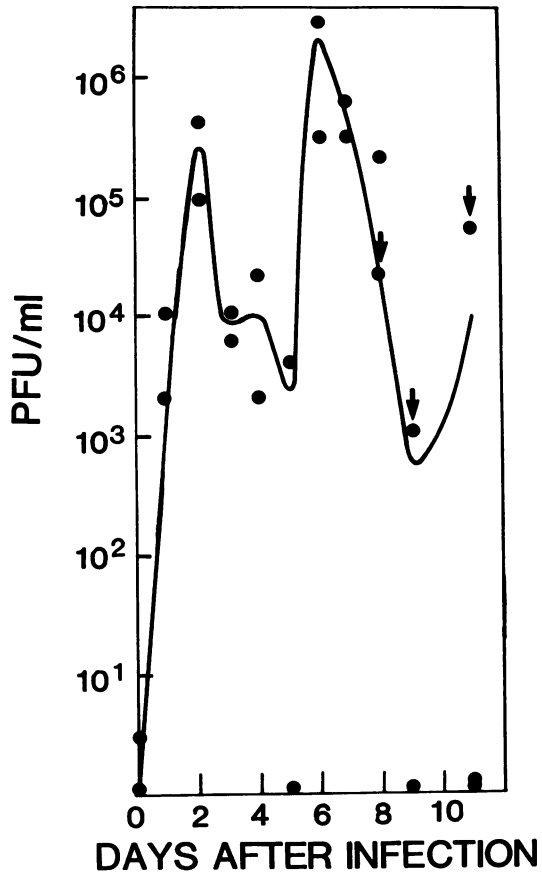

FIG. 3. Kinetics of VSV production in brains of mice. A total of 23 mice were inoculated intranasally with 10^8 PFU of standard VSV and 10^5 equivalents of DI-T particles. Each point represents the plaque assay from a 10% brain suspension from a single mouse sacrificed on the day indicated. The curve is drawn through the average. Arrows indicate assays that showed some evidence of DI particles.

cell-associated or soluble viral antigens as well as hybridization assays for nucleic acids. Another possible implication relates to transmission. If what is transmitted contains significant numbers of DI particles, their absolute numbers and ratio of standard virus to DI particles may determine the clinical pattern of disease in the contact recipient. This may explain why, during epidemics, many exposed individuals seroconvert without overt disease symptoms. Lastly, when DI particles of other viruses are mapped or sequenced, some contain functional genes (7). Cyclic production of these viruses and their DI particles may lead to instances with quantitative differences in the expression of certain genes. For example, if defective mutants contain genes responsible for avirulence and the standard virus contains lytic genes as well as transforming genes, then the transforming genes may only become effective when large numbers of defective mutants are present to prevent cell killing. Such a case was recently reported for equine herpesvirus (1). Therefore, variations in viral populations introduced by DI particles may determine whether viral infections lead to lytic or malignant disease.

To further determine whether such DI-particle-controlled cycling occurs during viral pathogenesis (10), it will be necessary to demonstrate by serial sampling during the course of a disease that there is overlapping cyclic production of standard and DI particles. Unfortunately, for many human viral pathogens, the range of DI particles and their genetic content have yet to be determined. This would be necessary before sensitive assays could be devised to measure the role of DI particles in human viral diseases. Nevertheless, the preliminary results reported here for mice coinfected with standard VSV and its DI particles indicate that at least in the mouse system, such cycling may be demonstrable.

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