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Neurovirulence of Murine Coronavirus JHM Temperature-Sensitive Mutants in Rats

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The murine coronavirus strain JHM is highly neurotropic in rats and has a marked tendency to cause demyelinating central nervous system diseases after intracerebral inoculation. The clinical diseases observed range from an acute encephalomyelitis occurring within 2 weeks postinfection to a subacute demyelinating encephalomyelitis developing several weeks or months postinfection. Uncloned wild-type virus induced both acute and subacute diseases, whereas cloned JHM virus grown in tissue culture caused only acute disease without the pronounced lesions of primary demyelination. In contrast, temperature-sensitive mutants selected from that clone were capable of inducing subacute demyelinating encephalomyelitis after prolonged incubation times. Viruses recovered from diseased animals were still temperature sensitive. Inoculation of temperature-sensitive mutants into suckling rats (age, 10 to 15 days) produced high rates of subacute demyelinating diseases running a more chronic course; these diseases often were not fatal. Those rats which did not show clinical signs frequently revealed inflammatory demyelinating lesions. These findings indicate that the rate and type of clinical disease are dependent on the neurovirulence of the virus mutant used for inoculation and the age of the animals at the time of infection.

Primary demyelination is a hallmark of certain human central nervous system (CNS) diseases which are associated with viral infection (7, 18). Little is known about the mechanisms by which the virus infection leads to myelin destruction. It has been suggested that the virus either destroys the oligodendroglia cells, which are responsible for the production of myelin, or induces an immunopathological reaction against myelin proteins (20, 21). The biological properties of the infectious agent are an important factor in this virus-host interaction, and attempts to characterize the infectious agent should contribute to the understanding of the pathogenesis of demyelinating diseases. In this context, the infection of rats by the JHM strain of coronavirus provides an interesting model. It has been shown that infection of weanling rats with wild-type JHM virus can lead to different courses of encephalomyelitis (9–11, 16). The onset of clinical disease can follow a short incubation time of 3 to 11 days (acute encephalomyelitis [AE]) or can develop more slowly for several weeks to months postinfection (p.i.) (subacute demyelinating encephalomyelitis [SDE]). AE, a fatal disease, is accompanied by complete paralysis, and necrotic lesions are detectable in all parts of the CNS. Virus particles are observed in neurons and glia cells. The clinical symptomatology of SDE is characterized by slight hind-leg paresis and ataxic gait. Pronounced demyelinating lesions are easily detectable and are located predominantly in the optic chiasma, midbrain, pons, cerebellum, and spinal cord. Neurons are often preserved, whereas glia cells are preferentially infected. Unlike the acute infection, SDE is not always fatal, and remyelination may occur after a prolonged disease process (10, 16). These different courses of disease suggest that properties of the virus might influence the outcome of the disease.

This possibility was investigated in the present study, and we describe here the neurotropic and virological characteristics of different virus preparations of murine coronavirus JHM, including temperature-sensitive (*ts*) mutants, which induce different CNS disease processes in rats after intracerebral infection under defined conditions.

MATERIALS AND METHODS

Virus. The murine coronavirus strain JHM has been propagated by mouse brain passages and was adapted and cloned on Sac(−) cells (19). For isolation of virus clones, virus was plaqued in agar overlay (final con-

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centration, 1%) containing Eagle minimal essential medium (MEM) and 5% fetal calf serum. After incubation for 2 days at 37°C, plaques were removed with a Pasteur pipette, suspended in 1 ml of MEM with 5% fetal calf serum, and snap-frozen before further passage. Cloning was performed by three plaque passages.

***ts* mutants.** Monolayers of Sac(−) cells were infected with cloned wild-type virus at a multiplicity of infection of 0.01 PFU per cell, and the virus was grown for 24 h at 34°C in the presence of 5 mM fluorouracil. The *ts* mutants were selected from mutagenized stocks either by selection of plaques which did not show growth at 39.5°C or by the replica method (4, 15) with microplates containing 60 20-μl wells. From each mutagenized stock only one mutant was selected, and this was further cloned by three plaque passages at 34°C.

Characterization of *ts* mutants. The efficiency of plaquing (EOP) was determined with a microplaque assay without agar overlay in 96-well microplates. The number of plaques produced at 34 and 39.5°C after 20 to 24 h incubation time was determined after staining with Giemsa.

To determine thermolability, virus suspensions were incubated in Eagle MEM with 5% fetal calf serum in a water bath at 39.5°C. At the start of the experiment and at different time points up to 4 h, samples were snap-frozen for virus titration. The percentages of wild-type and *ts* mutant virus surviving 4 h of incubation at 39.5°C were calculated. The thermolability index denotes the ratio between the percentage of surviving *ts* mutant and wild-type virus (thermolability index of wild type = 1).

To measure the production of infectious virus at the nonpermissive temperature (leakiness), two sets of cell cultures were infected with 0.1 PFU per cell and were incubated at 34 and 39.5°C. After an incubation time of 20 to 24 h, the infectious virus produced at each temperature was measured by plaque titration, and the ratio of the virus yield at 39.5°C to that at 34.0°C was calculated.

To determine the RNA phenotype of each mutant, the rate of virus-induced RNA synthesis was measured at 34 and 39.5°C. Monolayer cultures of Sac(−) cells (24-well cluster plates; diameter, 16 mm) were infected with 2 to 3 PFU per cell. To inhibit cellular RNA synthesis, actinomycin D (2 μg/ml) was added 2 hours p.i., and [^{3}H]uridine (5 μCi/ml; Amersham Corp., Arlington Heights, Ill., TRK 178) was added 3 h p.i. The assays were terminated, depending on the cytopathogenic effect of the wild-type virus, at 6 to 7 h p.i. for 39.5°C and at 8 to 11 h p.i. for 34°C. The cells were washed with ice-cold Hanks solution and lysed with 0.2% sodium dodecyl sulphate in distilled water, and the trichloroacetic acid-precipitable radioactivity was measured after filtration on GFC-glass fiber disks (Whatman, Inc., Clifton, N.J.). The RNA index was calculated by the method of Robb et al. (14). Mutants with an index of <0.1 were considered negative in RNA synthesis at 39.5°C. To obtain sufficiently high titers for infection under single-cycle growth conditions, several mutants were concentrated by polyethylene glycol precipitation as described previously (19) and were sonicated before infection.

Reisolation of virus from CNS tissue. Samples of brain and spinal cord were homogenized immediately after dissection and weight determination with a glass Dounce homogenizer, and a 10% (wt/vol) suspension was made in MEM containing 5% fetal calf serum and absorbed on monolayers of Sac(−) cells for 1 h at 34°C (0.3 ml per well of 24-well cluster plates; diameter, 16 mm). To measure the amount of virus reisolated (in 50% tissue culture infective doses per gram of tissue), dilutions of the tissue suspension were titrated as well. The cultures were washed twice with MEM and culture medium added. This contained 10% fetal calf serum, 2× vitamins, and amino acids. The number of wells in which syncytia developed was scored after 3 days of incubation at 34°C. Supernatants from positive cultures were pooled, and the EOP at 34 and 39.5°C was determined, or further passages were performed if the virus titer was too low for analysis.

TABLE 1. CNS diseases induced by uncloned and cloned JHM virus in weanling rats[a]

Virus	No. of rats		
	Diseased/total	Suffering from:	
		AE	SDE
Uncloned	38/92	20	18
Cloned	72/134	70	2

[a] Rats 21 to 25 days old were inoculated intracerebrally with 2×10^3 to 8×10^3 PFU of virus each.

Animals. Outbred specific pathogen-free rats (CHBB/Thom) were purchased from Thomae (Biberach, Germany). For infection, the rats were inoculated with a dispenser syringe into the left brain hemisphere and received 30 μl of virus suspension. Histological examinations were performed as previously described (9–11).

RESULTS

Neurovirulence of uncloned and cloned JHM virus. The development of two different types of CNS diseases in weanling rats after infection with uncloned JHM virus suggested that a heterologous virus population was used for inoculation. Therefore, experiments were carried out to plaque-purify JHM virus in tissue culture and test the neurovirulence of such clones in rats. Altogether, seven clones were selected, including large- and small-plaque variants. These clones differed in neurovirulence in rats and grew differently in tissue culture, but all of them induced only AE. The virus clone growing to the highest titer in tissue culture was used for this study. As documented in Table 1, uncloned JHM virus propagated by passages in mouse brain induced both AE and SDE in weanling rats. The number of animals developing AE or SDE varied considerably with the passage of the uncloned virus. In contrast, weanling rats infected with cloned, tissue culture-adapted virus revealed a high rate of AE, whereas SDE was only rarely observed. Inoculation of suckling rats with either uncloned or cloned wild-type virus resulted in fatal, rapidly progressing AE. The

TABLE 2. Biological properties of *ts* mutants

Virus	EOP[a]	Thermolability[b]	RNA synthesis (39.5°C)	Leakiness[d]
Wild-type Sac	1.2	1.00	1.000	1.1
*ts*1	2.6×10^{-3}	0.11	0.000	1.0×10^{-4}
*ts*5	7.7×10^{-2}	0.13	0.000	$<6.9 \times 10^{-3}$
*ts*6	1.0×10^{-1}	0.28	0.000	4.0×10^{-4}
*ts*16	$<1.2 \times 10^{-4}$	0.06	0.084	$<4.6 \times 10^{-3}$
*ts*18	4.8×10^{-4}	0.25	0.046	$<2.6 \times 10^{-4}$
*ts*22	4.0×10^{-2}	0.017	0.020	3.3×10^{-2}
*ts*24	1.0×10^{-1}	0.01	0.640	5.0×10^{-1}
*ts*31	1.0×10^{-3}	0.03	0.080	$<7.1 \times 10^{-4}$
*ts*33	$<5.7 \times 10^{-3}$	0.25	0.000	$<3.2 \times 10^{-3}$
*ts*36	2.7×10^{-5}	0.05	0.000	$<5.1 \times 10^{-4}$
*ts*41	4.4×10^{-4}	0.009	0.014	$<1.4 \times 10^{-3}$
*ts*42	1.6×10^{-5}	0.06	0.010	2.0×10^{-4}
*ts*43	1.3×10^{-5}	0.04	0.000	1.0×10^{-4}
*ts*59	1.0×10^{-4}	0.59	0.000	$<1.9 \times 10^{-5}$

[a] See Table 7, footnote *c*.

[b] Fraction surviving 4 h of incubation at 39.5°C relative to that of wild-type virus (index for wild-type virus = 1).

[c] [^{3}H]uridine incorporation in presence of actinomycin D. Infection with 2 to 3 PFU per cell. An index of <0.1 denotes a mutant which did not produce significant amounts of RNA at 39.5°C.

[d] Yield 20 to 24 h p.i. at 39.5°C versus yield at 34.5°C.

rate of mortality for both virus preparations was clearly dose dependent, but lower doses of cloned wild-type virus still induced only AE and not SDE.

To modify the neurovirulence of the tissue culture adapted cloned JHM virus, *ts* mutants were selected from virus stocks which were grown in the presence of fluorouracil as described above.

Biological properties of *ts* mutants. As summarized in Table 2, the mutants were characterized by determination of EOP at 34 and 39.5°C, by thermolability, and by leakiness. The EOP varied from 10^{-1} to 1.3×10^{-5}. All mutants were thermolabile, revealing an inactivation index of 0.28 to 0.009 relative to the wild-type virus (index, 1). Some of the mutants were quite leaky, and at the nonpermissive temperature

TABLE 3. Virulence of JHM wild-type virus and *ts* mutants for weanling and suckling rats

Virus	Dose (PFU/rat)	Effects on rats						
		Suckling (age, 3–5 days)				Weanling rats (age, 21–25 days)		
		No. diseased/ total	Incubation (days p.i.)	No. suffering from:		No. diseased/ total	Incubation (days p.i.)	Disease
				AE	SDE			
Cloned wild type	2.0×10^4	12/12	3–6	12	0	6/15	5–11	AE
*ts*1	1.4×10^6	14/15	3–6	14	0	1/43	180	SDE
*ts*5	1.5×10^3	12/12	3–6	12	0	0/23		
*ts*6	2.0×10^4	11/11	3–5	11	0	6/34	23–51	SDE
*ts*16	6.0×10^5	13/13	3–12	13	0	0/12		
*ts*18	1.4×10^4	7/15	11–19	3	4	1/12	74	SDE
*ts*22	2.0×10^4	8/16	9–19	2	6	0/28		
*ts*24	1.6×10^4	13/13	3–6	13	0	2/28	10, 31	AE, SDE
*ts*31	1.4×10^4	12/13	4–17	11	1	0/28		
*ts*33	2.0×10^4	8/11	10–32	6	2	0/16		
*ts*36	1.5×10^4	10/12	4–6	10	0	0/27		
*ts*41	3.0×10^4	15/15	4–9	15	0	1/27	23	SDE
*ts*42	1.0×10^4	15/15	6–10	15	0	2/41	40, 62	SDE
*ts*43	4.0×10^4	13/13	10–27	9	3	5/33	20–86	SDE
*ts*59	2.0×10^4	9/12	3–10	9	0	0/12		

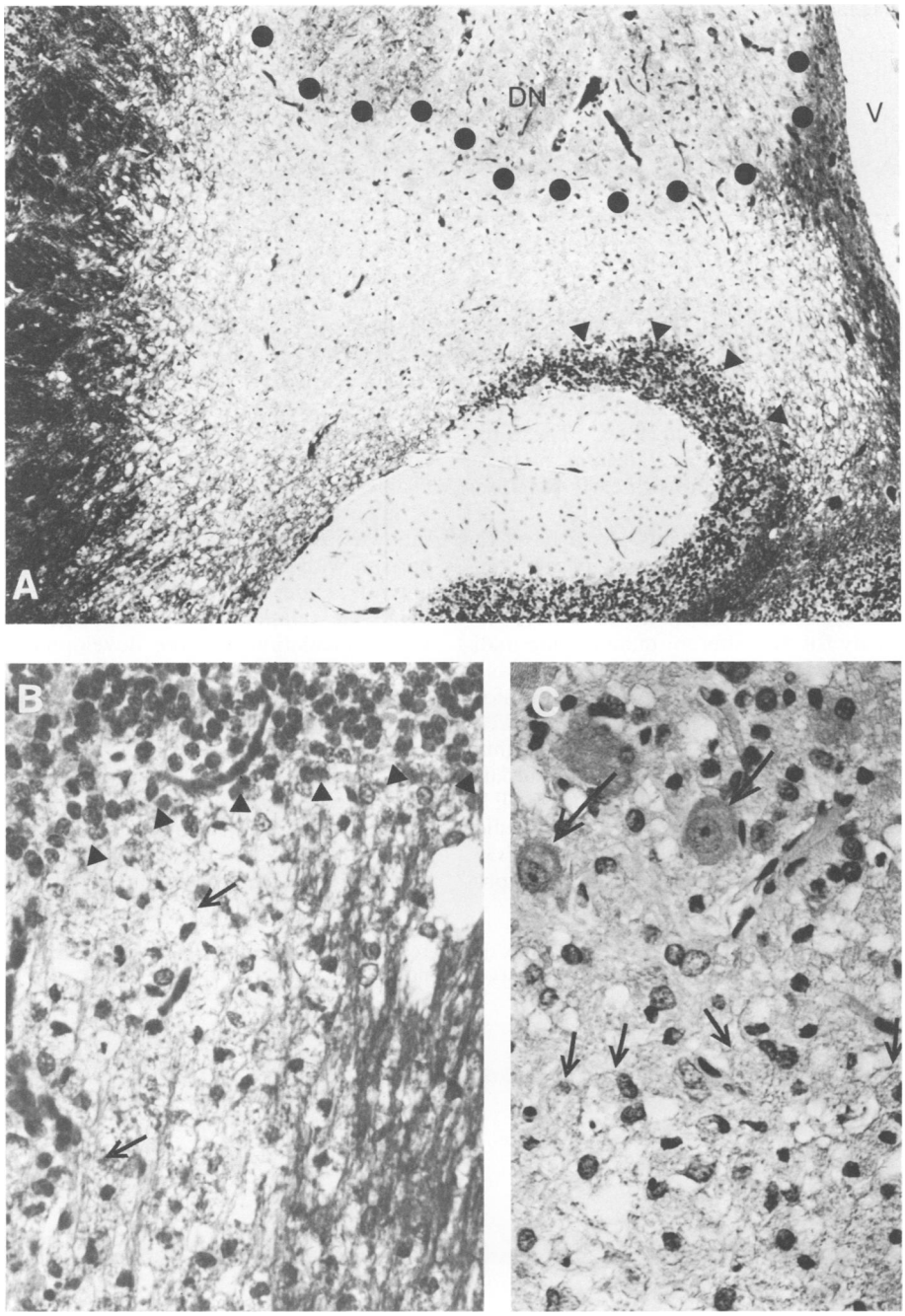

FIG. 1. Demyelinating plaque in the cerebellum of a rat with SDE 27 days p.i. The rat was infected with mutant *ts*6 at an age of 24 days. (A) Large, well-demarcated demyelinating plaque in the white matter. Note the well-preserved granular layer (▲) adjacent to the plaque. DN, Dentate nucleus (●); V, ventricle. Hematoxylin-eosin and luxol fast blue double staining; magnification, ×100. (B) Higher magnification from the edge of the plaque, showing infiltration of macrophages (arrows) to left and still-preserved white matter to right. Neuronal perikarya of granular layer (▲) are well preserved. Hematoxylin-eosin and luxol fast blue double staining; magnification, ×360. (C) Well-preserved neurons in the dentate nucleus (large arrows) and infiltration of macrophages (small arrows). Hematoxylin-eosin staining; magnification, ×572.

TABLE 4. Dose dependence of disease rate in suckling rats (age, 3 to 5 days) inoculated with mutant *ts*6

Virus dose (PFU/rat)	No. diseased/ total	Incubation (days p.i.)	Disease
2×10^4	12/12	3–6	AE
2×10^3	29/33	5–8	AE
2×10^2	4/19	5–8	AE
2×10^1	0/21		AE

they produced 10 to 20% of the yield which was obtained at 34°C. At the nonpermissive temperature, no synthesis of virus-induced RNA was detectable. The only exception observed was with *ts*24, a mutant which is also very leaky and produces infectious virus at 39.5°C. These observations suggest that the *ts* lesions of these mutants may be associated with early functions of virus replication.

Neurovirulence of *ts* mutants. As a basis for further investigations, the capacity of each mutant to induce AE and SDE was tested. Since the maximum titers obtained in tissue culture differed widely for the different mutants, the undiluted stock virus was inoculated intracerebrally into suckling and weanling rats to obtain information on neurovirulence first. All mutants were pathogenic for suckling rats but differed significantly in their virulence (Table 3). The mutants *ts*1, *ts*5, *ts*6, *ts*24, *ts*36, and *ts*59 induced AE in suckling rats much as the wild-type virus did, whereas the mutants *ts*16, *ts*18, *ts*22, *ts*31, *ts*33, *ts*42, and *ts*43 tended to induce SDE after prolonged incubation times. Weanling rats were, however, much less susceptible to CNS disease after infection with *ts* mutants. Only mutants *ts*1, *ts*6, *ts*18, *ts*24, *ts*41, *ts*42, and *ts*43 caused disease with an incubation time of up to several months (Table 3). Neuropathologically, these animals revealed changes of SDE. As in the morphological changes obtained with uncloned virus (9, 10), plaques of primary demyelination were detected in the white matter of the CNS. An example of a marked demyelinating lesion is shown in Fig. 1.

All mutants which killed more than 50% of suckling rats were further compared at lower doses. The percentage of diseased animals decreased in parallel to the dose of infectious virus inoculated, but suckling animals still developed acute fatal disease after a short incubation period of about 3 to 6 days, as exemplified by *ts*6 (Table 4).

Since the rate of SDE observed in weanling rats was relatively low (about 2 to 5%) in comparison with the rates of AE and SDE in suckling rats, experiments were carried out to investigate the relationship between age at time of virus inoculation and the development of the different CNS diseases.

Age-dependent development of CNS diseases. Litters of rats aged 4, 10, and 15 days were inoculated with 4×10^3 PFU per rat of either wild-type virus or *ts* mutants. Cloned wild-type virus induced only acute diseases regardless of the age at the time of inoculation (Table 5). The *ts* mutants, however, revealed a striking capability to cause SDE dependent on the age of the

TABLE 5. Development of CNS disease in rats of different ages at time of inoculation with JHM wild-type virus or *ts* mutants

Age at infection (days ± 1)	Virus[a]	No. diseased/ total	Incubation (days)	Survival[b]	No. of rats suffering from:	
					AE	SDE
4	Wild type	7/10	3–5	None	7	0
	*ts*1	14/15	3–5	None	14	0
	*ts*6	11/12	5–8	None	11	0
	*ts*42	12/14	5–10	None	12	0
	*ts*43	26/26	10–17	5/26	17	9
10	Wild type	8/10	3–6	None	8	0
	*ts*1	8/13	5–17	3/8	5	3
	*ts*6	32/39	5–17	9/32	23	9
	*ts*42	14/17	5–23	2/14	7	7
	*ts*43	23/43	14–147	11/23	0	23
15	Wild type	7/12	4–11	None	7	0
	*ts*1	0/10				
	*ts*6	13/37	7–25	8/13	4	9
	*ts*42	0/10				
	*ts*43	1/12	92		0	1

[a] Dosage, 4×10^3 PFU per rat.
[b] No. survivors/no. diseased.

TABLE 6. Neuropathological lesions in virus-infected clinically healthy rats dissected 30 to 40 days p.i.

Virus	Dose (PFU/rat)	Age at infection (days ± 1)	Rats with lesions/ rats dissected
*ts*1	4×10^3	10	4/10
	4×10^3	24	0/8
*ts*6	2×10^4	15	2/5
	2×10^4	24	1/18
*ts*42	4×10^3	10	5/8
	4×10^4	24	1/5
*ts*43	4×10^3	15	3/8
	4×10^4	24	3/15

rats at the time of virus inoculation. As the age at the time of infection increased, higher percentages of animals developed SDE and recovered from disease, whereas AE was observed less frequently. In general, those animals which developed disease within 3 to 12 days p.i. revealed AE, whereas clinical signs observed later than 12 days resulted from SDE. It is especially remarkable that about 14 to 62% of rats inoculated at the age of 10 to 15 days with the mutants *ts*1, *ts*6, *ts*42, and *ts*43 survived SDE. These animals either recovered completely or maintained clinical signs, for example, hind-leg paresis or ataxic gait.

Clinically silent lesions. The possibility that infected rats without clinical signs of disease might possess silent neuropathological lesions was tested in the following experiments. Groups of rats aged 10, 15, or 24 days were inoculated with different *ts* mutants, and the experiments were terminated 30 to 40 days p.i. by the dissection of all rats which had not developed clinical signs within that period. As shown in Table 6 for the mutants *ts*1, *ts*6, *ts*42, and *ts*43, clinically silent lesions were frequently detected in brains and spinal cords if the rats had been infected at the age of 10 to 15 days. However, infection at the age of 24 days resulted in a reduced frequency of neuropathological changes (Table 6). These lesions consisted often of demyelinating plaques and infiltrations of mononuclear cells (Fig. 2).

Demyelinating lesions can therefore be present without clinically recognizable disease, but these changes occurred only at low frequency if rats were infected at an age of reduced susceptibility for clinical disease.

Temperature sensitivity of viruses reisolated from diseased animals. In clinically healthy animals, virus could only be isolated from homogenates of brain and spinal cord within 2 weeks p.i. However, in diseased animals it was always possible to reisolate virus from CNS tissue irrespective of the time between virus inoculation and the onset of disease (as shown in Table 7 for rats infected with *ts*43). Rats were dissected within 2 to 3 days after the onset of clinical signs. In most cases, the amounts of infectious virus isolated from spinal cords were significantly higher than those obtained from brain tissue. Reisolated viruses were still *ts* and were therefore not revertants to the wild type. Inoculation of some reisolates into suckling and weanling rats revealed unaltered neurovirulence compared with the original *ts* mutant.

DISCUSSION

Murine coronavirus JHM has been shown to be a neurotropic agent which causes demyelinating CNS diseases in mice and rats. Acute fatal diseases were usually observed in mice. A chronic infection could also occur with the development of demyelinating lesions, especially if *ts* mutants of JHM virus were used for inoculation (5, 6, 14, 17). However, the lesions in chronically infected mice were minute and did not lead to a clinically recognizable disease. In contrast, JHM infection of rats was followed by CNS disease processes with different clinical courses and neuropathological changes (9–11, 16). Acute as well as chronic CNS infections could be observed, and these infections were characterized by marked pronounced neuropathological and clinical changes. This correlation between CNS lesions and clinical symptoms in infected rats provides a convenient experimental approach to the analysis of the underlying disease mechanisms.

The induction of AE as well as SDE by the wild-type JHM virus within the same litter suggests that the virus population was heterogeneous and could consist of variants of different neurovirulence. However, attempts to separate these populations into those which caused only acute or subacute disease were not successful. Tissue culture adaptation of JHM virus propagated in mouse brain resulted in virus clones which after infection of rats led predominantly to acute diseases, independent of the amount of virus inoculated and the age of the animals at the time of infection. The more virulent and cytopathogenic JHM virus population was apparently preferentially isolated in tissue cultures susceptible to JHM virus. Therefore, experiments were carried out to obtain JHM virus mutants which could induce high rates of SDE.

Mutants of JHM virus were isolated after mutagenesis with 5-fluorouracil and were selected for temperature sensitivity. These mutants differed in biological properties such as thermolability and EOP and produced lower yields of infectious virus than the wild-type virus did in

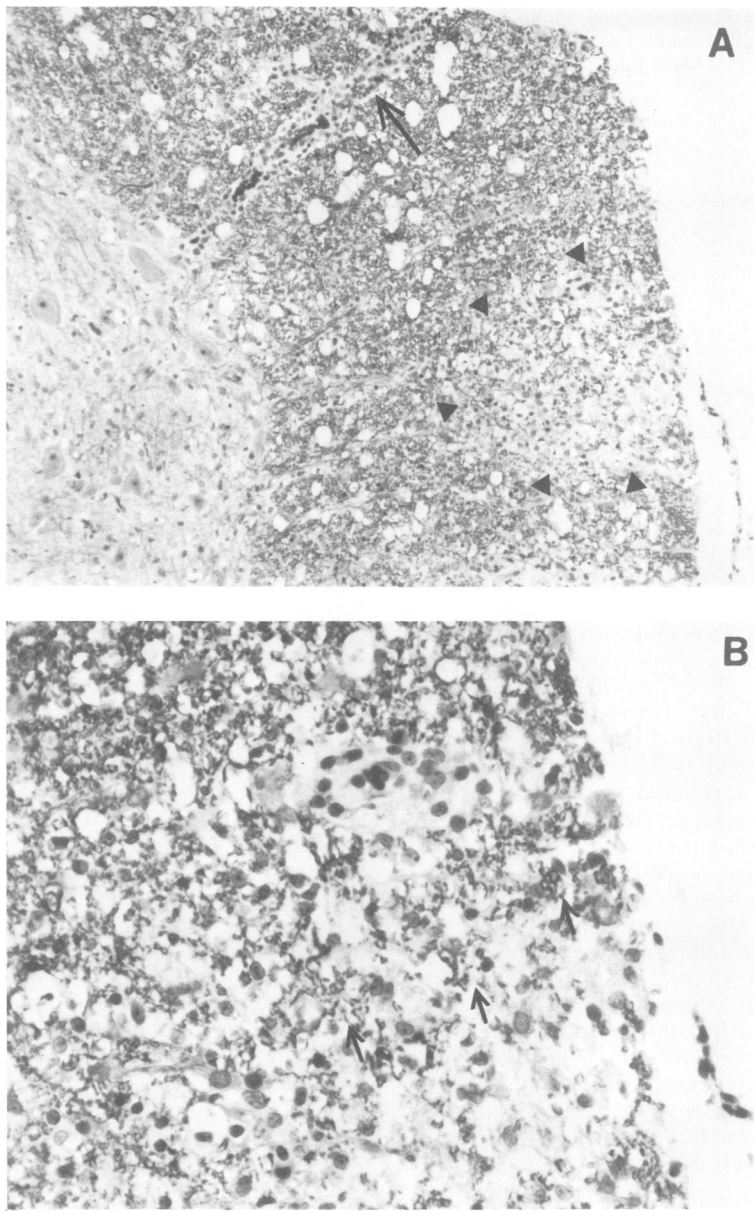

FIG. 2. Clinically silent demyelinating lesion in the spinal cord 34 days p.i. The rat was infected at an age of 11 days with mutant *ts*43. (A) Small plaque (▲) accompanied by mild perivascular cell infiltration (arrow). Hematoxylin-eosin and LFB double staining; magnification, ×144. (B) Higher magnification of the same plaque. The edge of the plaque is not sharply demarcated. Myelin structure is still recognizable within the plaque (arrows). Hematoxylin-eosin and luxol fast blue double staining; magnification, ×360.

tissue culture. The mutants obtained were also RNA negative at the nonpermissive temperature. The *ts* lesions of these mutants therefore probably affect either the early steps of replication before RNA synthesis or the RNA synthesis itself.

In animal experiments, these mutants showed interesting differences in their neurovirulence. Infection of suckling rats with the majority of *ts* mutants resulted in AE, as was also observed with the cloned wild-type virus. Some *ts* mutants showed a distinct pathogenicity for suckling rats and failed to induce disease in weanling rats. Several *ts* mutants led to a high incidence of SDE in 10-day-old suckling rats. In general, the percentage of animals with recognizable

TABLE 7. Temperature sensitivity of reisolated virus from rats infected with mutant *ts*43[a]

Isolate	Onset of clinical disease (days p.i.)	Amt of virus reisolated from[b]		Temperature sensitivity of reisolated virus (EOP)[c]
		Brain	Spinal cord	
*ts*43-1	45	4.2×10^2	1.2×10^4	$<1.3 \times 10^{-4}$
*ts*43-2	48	$<1.6 \times 10^1$	1.5×10^3	$<1.5 \times 10^{-4}$
*ts*43-3	50	7.7×10^1	1.0×10^4	$<6.7 \times 10^{-3}$
*ts*43-4	50	3.2×10^3	1.0×10^4	$<5.0 \times 10^{-4}$
*ts*43-5	65	2.5×10^2	6.2×10^3	$<5.0 \times 10^{-3}$
*ts*43-6	76		3.3×10^2	$<2.0 \times 10^{-2}$
*ts*43-7	90	3.6×10^1	4.2×10^2	$<4.0 \times 10^{-3}$

[a] Rats aged 9 to 11 days were inoculated with 4×10^3 PFU of virus each.
[b] Measured as 50% tissue culture infective doses per gram of tissue.
[c] EOP = (PFU at 39.5°C)/(PFU at 34°C). EOP of *ts*43 used for inoculation was 1.3×10^{-5}.

symptoms seen after the inoculation of JHM *ts* mutants decreased with the age of the rat, and the CNS disease process changed from an acute to a subacute form. In weanling rats only SDE was produced after infection with *ts* mutants. For each mutant, the susceptibility of the animal and the type of CNS disease varied and was strictly age dependent. The viruses reisolated after prolonged incubation times were still *ts*, indicating that the development of clinical disease did not result from reversion to wild type, as has been described for Sindbis virus mutants in mice (1).

These observations underline the role of host defense mechanisms in the development of CNS diseases caused by JHM virus. Age-related resistance to virus infections has been associated with metabolic and hormonal changes, inhibitory substances, interferon production, and differentiation of cells (3, 12). In addition, the susceptibility of macrophages could play a major role since it has been shown that the restriction of virus replication in cultured macrophages is increased with the age of the animal (8). Moreover, it has also been found that the outcome of JHM infection in mice depends on the maturation of the immune system (13). These factors and the neurovirulence of the JHM virus used for infection determine the outcome of the CNS disease process.

The differences in pathogenicity and capacity to induce demyelination observed among *ts* mutants of JHM virus used in our experiments could be due to an altered cellular tropism within the CNS. It has been shown by Knobler et al. (6) that replication of a JHM virus *ts* mutant in mice was demonstrable primarily in oligodendrocytes and very rarely in neuronal cells. Differences in cell tropism between wild-type virus and a *ts* mutant have also been observed in infections of primary mouse brain cell cultures (2). This selective vulnerability of oligodendroglia cells to JHM *ts* mutants may be one important factor in the development of demyelination. On the other hand, the high rate of clinically silent lesions in rats dissected 30 to 40 days p.i. suggests that in many animals the CNS infection was arrested by mechanisms which are not yet understood. This CNS infection can be controlled by the host, and no clinical disease develops.

The availability of JHM *ts* mutants has made it possible to induce in rats a high rate of SDE. It is hoped that infection of inbred rats with *ts* mutants of JHM virus will help to define the viral and host factors which determine the CNS disease process.

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