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*ts*1, a Paralytogenic Mutant of Moloney Murine Leukemia Virus TB, Has an Enhanced Ability to Replicate in the Central Nervous System and Primary Nerve Cell Culture

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A temperature-sensitive mutant of Moloney murine leukemia virus TB (MoMuLV-TB), *ts*1, which is defective in intracellular processing of envelope precursor protein (Pr80^{env}), also possesses the ability to induce hind-limb paralysis in infected mice. To investigate whether *ts*1 has acquired neurotropism and to determine to what extent it can replicate in the central nervous system, we compared viral titers in the spleen, plasma, spinal cord, and brain throughout the course of infection of mice infected with *ts*1 and parental wild-type (wt) MoMuLV-TB. In both the *ts*1- and wt-inoculated mice, the concentrations of infectious virus recovered from the plasma and spleen increased rapidly and reached a plateau by 10 days postinfection (p.i.). In contrast, virus concentrations in the spinal cord and brain of *ts*1-inoculated mice increased gradually and reached a titer comparable to that in the spleen and exceeding that in the plasma only at 25 to 30 days p.i. At this time, the virus titer was ~200× greater in *ts*1-infected spinal cord tissue and ~20× greater in *ts*1-infected brain tissue than in the same wt-infected tissues. Paralysis became evident at 25 to 30 days p.i. in *ts*1-inoculated mice, whereas the wt-inoculated mice were normal. In addition, a substantial amount of Pr80^{env} was detected in the spinal cords of *ts*1-inoculated mice compared with that found in the spinal cords of wt-inoculated mice. The infectious virus isolated from *ts*1-infected nerve tissue was found to possess the characteristic phenotype of the *ts*1 virus. Microscopic lesions of *ts*1-inoculated mice at 30 days p.i. consisted of vacuolar degeneration of motor neurons and spongy change of white matter in the brain stem and spinal cord. Similar but less severe lesions were observed in wt-inoculated mice. With primary cultures of central nervous system tissue we showed that *ts*1 can infect and replicate in both neuron and glial cells. In contrast, although wt MoMuLV-TB replicated in glial cell-rich culture, viral replication was barely detectable in neuron-rich culture.

Certain inbred strains of mice when inoculated with a unique group of temperature-sensitive (*ts*) mutants of Moloney murine leukemia virus TB (MoMuLV-TB), *ts*1, *ts*7, and *ts*11, develop hind-limb paralysis (13, 24). For paralysis to develop, the mice must be inoculated during the first few days of life. Clinical signs first appear at young adulthood; the disease progresses rapidly and is fatal. This paralytogenic response to the *ts* mutants is in contrast to the induction of T-cell lymphoma by the parental wild-type (wt) MoMuLV-TB in the same mouse strains.

Similar paralytic diseases have been induced by the Cas-Br-E strain of MuLV, which was initially isolated from the brain of a paralyzed feral mouse (for a review, see reference 7), and by a rat-passaged Friend MuLV which was isolated from a paralyzed Fischer rat (10). The chain of events that culminate in paralysis in these paralytogenic-virus-inoculated mice is still not well defined.

The paralytogenic *ts* mutants of MoMuLV-TB cause paralysis in 100% of infected mice, with a shorter latent period than that of other known strains of paralytogenic MuLVs. Because these mutants were derived from a non-paralytogenic parent, the genomic change(s) in the mutants which confers on the virus the paralytogenic potential can be identified. In addition, differences in phenotypic expression at the cellular level, both in vitro and in vivo, can be compared between mutant- and wt-inoculated cells. Gene swapping experiments in which genome fragments are interchanged between any one of these mutant and wt strains can be performed so that the altered phenotypic expression and the appearance of clinical signs can be correlated with a particular subgenomic sequence.

The paralytogenic *ts* mutants share a common characteristic in their inability to process efficiently the *env* precursor polyprotein Pr80^{env} to gp70 and p15E. This results in the accumulation of Pr80^{env} protein in infected cells and the production of virions with reduced amounts of gp70 and p15E (24). Although this effect is most pronounced at the restrictive temperature (38 to 39°C), the ratio of Pr80^{env} to gp70 is relatively greater in *ts*1-infected cells than in wt-infected cells even at the permissive temperature (34 to 36°C).

We recently identified a 1.6-kilobase-pair *Hin*dIII-*Bam*HI fragment in the *ts*1 genome comprising the 3′ end of *pol* and 5′ end of *env* that is responsible for the inability of *ts*1 to process Pr80^{env} intracellularly. Replacement of this 1.6-kilobase-pair sequence of *ts*1 with the homologous sequence of wt MoMuLV-TB prevents the induction of paralysis in infected mice (26). These findings indicate that the failure to process Pr80^{env} may play a role in the induction of paralysis.

The experiments described in this paper are part of a study designed to gain an understanding of the molecular pathogenesis of *ts* mutant-induced paralytic disease. A crucial question is whether the paralytogenic mutants are better able to enter and replicate in the cells of the central nervous system (CNS) and what effect they have on these cells. A prototype of the paralytogenic group of mutants, *ts*1, was used to infect neonatal CFW/D mice in these studies. Our findings show that *ts*1 can infect and replicate much more efficiently in the CNS than does wt virus. Furthermore, *ts*1 virus infection of the CNS resulted in neuronal degeneration

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which was similar to but much more pronounced than that found in wt-inoculated mice.

MATERIALS AND METHODS

Cells. A thymus-bone marrow (TB) cell line derived from CFW/D mice (1) was used for propagation of both MoMuLV and the *ts*1 mutant as described by Wong et al. (23). 15F, a nontransformed, nonproducer, sarcoma-positive, leukemia-negative (S^+L^-) cell line (28), was used to determine virus titers. All cell lines were cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum and containing 50 μg of gentamicin per ml of medium.

Virus and virus assay. The strain of MoMuLV-TB used in this study was isolated as described by Wong et al. (23) from the tissue extract of a sarcoma produced in a MoMuLV-infected BALB/c mouse provided by Moloney. Since its isolation, it has been propagated in TB cells. MoMuLV-TB has been single-virus single-cell cloned on several occasions. Clone LV30, used in the present studies, is one of the isolates obtained in the most recent clonal isolation (27).

*ts*1 is a spontaneous *ts* mutant of MoMuLV-TB isolated as described by Wong and co-workers (23). The *ts*1 used in the present studies was also recently purified by cloning TB cells infected with *ts*1 at a multiplicity of infection of 0.01.

The modified 15F assay used for determining virus titer has been described previously (25).

Mouse strain. The inbred CFW/D mice used in this study were bred by techniques outlined by Bennett and Vickery (2) and Inglis (9) from stock kindly provided by J. K. Ball and J. A. McCarter, University of Western Ontario. The breeding colony and the inoculated animals were maintained as described previously (27).

Viral infectivity assay from inoculated mice. CFW/D mice were inoculated within 24 h after birth intraperitoneally with 0.1 ml of virus suspension containing 10^6 to 10^7 infectious units (IU)/ml. Mice were sacrificed at 5, 10, 15, 20, 25, and 30 days of age, and plasma, spleen, brain, and spinal cord extracts were assayed with 15F cells for infectious virus.

Metabolic labeling and immunoprecipitation. Newborn mice inoculated with MoMuLV-TB or *ts*1 were sacrificed 5, 10, 15, 20, 25, and 30 days after inoculation. For each time point, the brains, spleens, and spinal cords were removed and pooled from two to three mice. The organs were washed with MEM and minced. Detached cells were passed through a 100-mesh tissue sieve (Bellco Glass, Inc.) and resuspended in 2 ml of leucine-free MEM in a 60-mm petri dish. Cells were labeled with 250 μCi of [^{3}H]leucine (New England Nuclear Corp.; specific activity, 400 Ci/mmol) at 39°C for 4 h. After being labeled, cells were removed from the petri dish, washed three times with phosphate-buffered saline, and lysed in RIPA buffer (10 mM Tris [pH 7.2], 150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate, 1% aprotinin). Portions of lysates (2×10^6 cpm) were immunoprecipitated with monospecific goat anti-MuLV gp70 (obtained through the Office of Program Resources and Logistics, Viral Oncology, National Institutes of Health) followed by a *Staphylococcus aureus* suspension (Pansorbin; Calbiochem-Behring) and analyzed in a linear 7.5 to 17.5% gradient slab gel as described previously (24, 25).

Primary cultures. Spinal cords were aseptically dissected from 10-day gestation fetal mice and dissociated mechanically by using a narrowed orifice pipette (6) in DMEM (GIBCO Laboratories) supplemented with 10% fetal bovine serum and 10% heat-inactivated horse serum (DMEM 10/10). Cell viability was determined by erythrosine B dye exclusion (17). The cell suspensions were plated onto 16-mm glass cover slips precoated with poly-L-lysine (type 1-B, molecular weight 90,000; Sigma Chemical Co.) at a concentration of 10^5 cells per cover slip in a 35-mm^2 culture plate (Costar) (22). After incubation at 37°C with 9% CO_2 for 48 h, the medium was removed and DMEM 10/10 with 12 μg of 5-fluorodeoxyuridine and 3 μg of uridine per ml was added back for a period of 24 h (8). The plates were then divided into two groups: one group received DMEM 10/10 with 5 μg of Polybrene (Sigma) per ml, and the other received Sf-Mod-DMEM with the same amount of Polybrene. Sf-Mod-DMEM is the modified DMEM of Kaufman and Barrett (11) with the addition of serum-free components as described by Bottenstein and Sato (3) and Skaper et al. (19). We found that this medium enhanced neuron growth and survivability after treatment with DMEM 10/10 with 5-fluorodeoxyuridine. After 48 h, each of the two groups of cells was either infected with *ts*1 or MoMuLV-TB with a multiplicity of infection of 3 or kept as a control. The viral inoculum (0.5 ml) was allowed to remain on the cultures for 1 h, and the appropriate medium was replaced afterwards. Twenty four hours postinfection (p.i.), the medium was again removed, and the plates were washed twice with DMEM before the appropriate maintenance medium was replaced. Thereafter, the medium was changed every 72 h. Eighteen days from the establishment of the culture, supernatant from each plate was assayed by using the 15F cell assay. Protein analysis of the processing of $Pr80^{env}$ was done in the cell cultures maintained with DMEM 10/10. However, because of the low number of nerve cells per plate ($\sim 10^3$ cells) after the selection procedures, protein analysis of the processing of $Pr80^{env}$ was not done in cultures maintained with Sf-Mod-DMEM.

Histopathology. At 30 days p.i., *ts*1- and MoMuLV-TB-inoculated mice and control mice were placed under deep anesthesia, perfused with 0.5 U of heparin per ml with 5% glucose in phosphate-buffered saline (pH 7.3), and then fixed by perfusion with 10% Formalin in phosphate-buffered saline (pH 7.3). The brain, spinal cord, spleen, liver, kidney, and thymus were stored in the above fixative before embedding. Tissues were decalcified, dehydrated, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E).

RESULTS

Virus replication in newborn mice. Infectious virus was detected in the plasma of newborn CFW/D mice 24 to 48 h p.i. By day 5, comparable plasma viremia (5×10^2 to 1×10^3 IU/ml) developed in both *ts*1- and MoMuLV-TB-inoculated mice. This was followed by an exponential increase in viremia up to 10 days p.i. A slight decrease in virus titer was observed 25 to 30 days p.i. (Fig. 1). At this time, signs of paralysis were generally observed in the mice infected with *ts*1 virus, whereas mice infected with wt virus were normal.

Comparable titers of infectious virus were also recovered by day 5 from spleens of mice inoculated with *ts*1 and wt viruses. The virus titer increased exponentially between 5 and 10 days after inoculation. Thereafter, virus titers in spleens of *ts*1- and wt-inoculated mice persisted at similar levels throughout the course of infection. Virus titers in the spleens of both *ts*1- and wt-infected mice during this period were about 50-fold greater than that in the plasma.

The increase in virus titer in spinal cord and brain tissues for both *ts*1- and wt-infected mice appeared to be slower

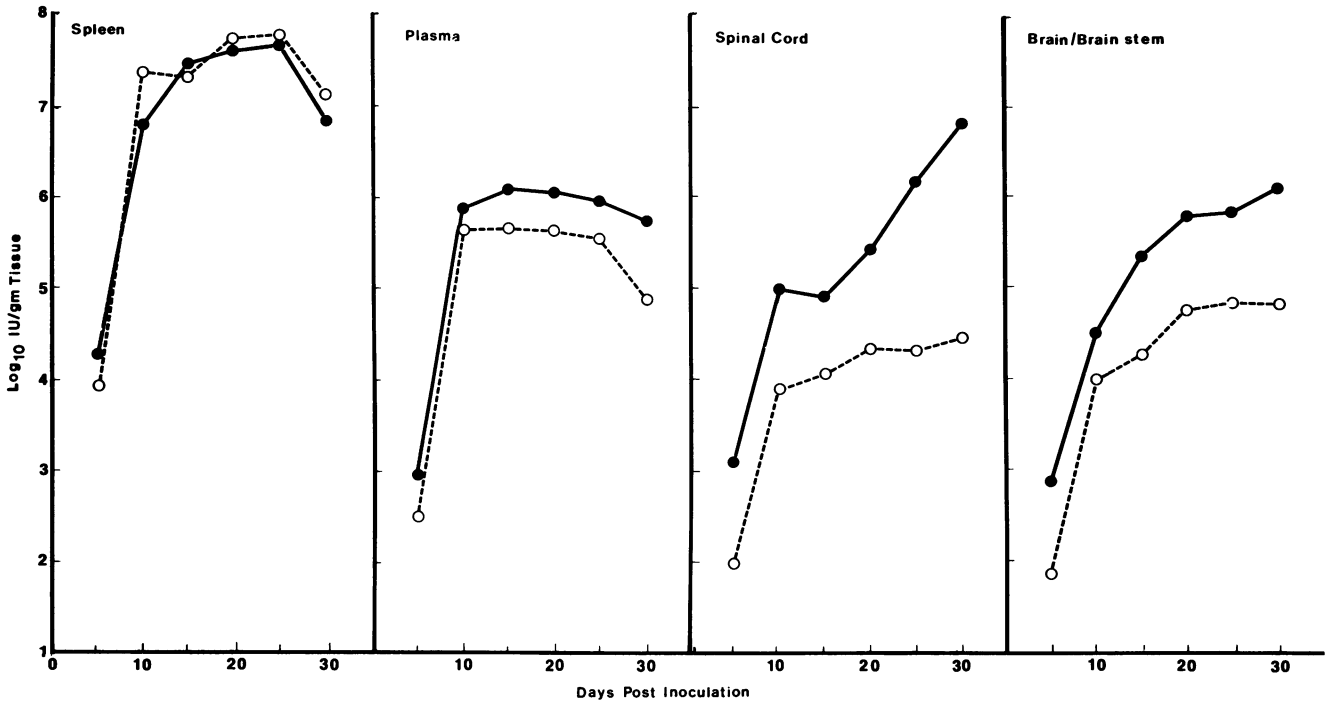

FIG. 1. Virus replication in various organs of MoMuLV-TB- and *ts*1-infected CFW/D mice. Newborn CFW/D mice were inoculated intraperitoneally with 0.1 ml of virus suspension containing 10^6 to 10^7 IU/ml. Virus-infected mice were sacrificed at 5, 10, 15, 20, 25, or 30 days of age, and serum, spleen, brain, and spinal cord extracts were assayed for viral infectivity. Symbols: ●, *ts*1 titer; ○, MoMuLV-TB titer.

than that found in the spleen and plasma. However, in contrast to the other tissues, infectious virus titers from the spinal cord and brain homogenates were significantly higher in *ts*1-inoculated mice than in mice inoculated with wt virus. By day 10 p.i., the virus titer from the spinal cord of wt-inoculated mice began to level off, as observed previously for both *ts*1 and wt virus titers in the spleen and plasma. In contrast, the virus titer in the spinal cord of mice inoculated with *ts*1 continued to increase until 30 days p.i. A similar pattern was observed with *ts*1 virus titers obtained from the brain homogenates, although it was not as marked as in the spinal cord. By 25 days p.i., *ts*1 virus titers in the spinal cord and brain were higher than those found in the plasma. By 30 days, the titer of *ts*1 virus recovered from the spinal cord was about 14-fold the titer of the virus recovered from the plasma, indicating that the virus recovered from the CNS was not from contaminating plasma.

When paralysis was evident in *ts*1-inoculated mice (about 25 to 30 days p.i.), the concentrations of virus in the spinal cord and the brain were ~200- and 20-fold greater than those in the respective tissues of mice inoculated with the wt virus.

Characteristics of the virus recovered from the CNS of infected mice. Temperature sensitivity of the virus recovered from the various tissues of mice 30 days p.i. was determined by 15F assays at both the permissive (34°C) and restrictive (39°C) temperatures. All tissue homogenates from *ts*1-inoculated mice had virus titers which were at least 1,000× greater at 34°C than at 39°C (Table 1). This indicated that the virus obtained from the tissues of *ts*1-inoculated mice retained the *ts* phenotype of *ts*1 virus and was therefore most likely the progeny of *ts*1 virus. In contrast, virus obtained from similar tissues of mice at 30 days p.i. with wt virus had a difference in titer of only 1.1- to 1.4-fold at the two temperatures.

Another phenotypic expression of *ts*1 virus was inefficiency in the processing of the precursor $Pr80^{env}$, resulting in intracellular accumulation of this polyprotein. To determine whether this phenomenon also occurred in cells in the spinal cord and brain, we examined the relative amounts of $Pr80^{env}$ and gp70 in cells isolated from these tissues at different times p.i. with *ts*1 virus.

At 25 days p.i. (Fig. 2), when the clinical symptom of hind-limb paralysis became apparent, a considerable amount of $Pr80^{env}$ was observed in cells obtained from the spinal cords of *ts*1-inoculated mice, whereas no gp70 was detectable. This indicated that the $Pr80^{env}$ was synthesized in these cells but was not processed into gp70 and p15E. Similar observations were noted in the cells from spinal cord and brain homogenates of mice sacrificed at 30 days p.i. with *ts*1 virus. On the other hand, little or no detectable $Pr80^{env}$ or gp70 was found in the spinal cord or brain of wt-inoculated mice at 25 or 30 days p.i. This observation was in agreement with our findings that *ts*1 titers were much greater than wt titers in the CNS at 25 and 30 days p.i. This was also consistent with the finding that the virus recovered from the spinal cords and brains of *ts*1-inoculated mice retained its *ts* phenotype.

Preliminary histopathologic studies. Control, wt-inoculated, and *ts*1-inoculated mice were submitted for necropsy at

TABLE 1. Comparison of titers of viruses recovered from tissue homogenates of mice 30 days p.i. and assayed at 34 and 39°C

Tissue	Titer at 34°C (IU/g)/titer at 39°C (IU/g) for	
	MoMuLV-TB	*ts*1
Plasma	1.4	1.5×10^3
Spleen	1.2	2.3×10^3
Brain	1.4	$>1 \times 10^3$
Spinal cord	1.1	$>1 \times 10^3$

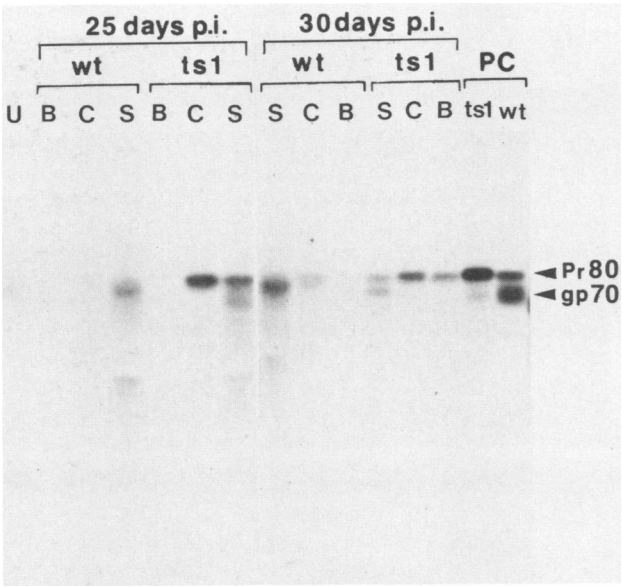

FIG. 2. Newborn mice were inoculated with MoMuLV-TB or *ts*1 and were sacrificed 25 and 30 days after inoculation. For each time point, the brains, spleens, and spinal cords were pooled from two to three mice and minced with 2 ml of leucine-free MEM in a 60-mm petri dish. Cells were labeled with 250 μCi of [^{3}H]leucine at 37°C for 4 h. After being labeled, cells were removed from the petri dish, washed three times with PBS, and lysed in RIPA buffer. Portions of lysates (2×10^6 cpm) were immunoprecipitated with goat anti-MuLV gp70 followed by an *S. aureus* suspension and analyzed in a linear 7.5 to 17.5% gradient slab gel as previously described (24, 25). Gels were fluorographed and exposed to X-ray film at −70°C. Abbreviations: U, uninfected control spleen; B, brain; C, spinal cord; S, spleen; PC, primary nerve cell culture in DMEM 10/10.

30 days of age. In contrast to control and wt-inoculated mice, the appendicular musculature of *ts*1-inoculated mice was atrophied. No significant gross lesions were observed in other tissues. Microscopic lesions were confined to the CNS in both *ts*1- and wt-inoculated mice. These lesions were more marked in *ts*1-inoculated mice and consisted of symmetrical vacuolar degeneration of motor neurons and of spongy change in myelinated areas (Fig. 3). Spongy change of myelinated areas occurred in the brain stem, cerebellar peduncles, and lateral and ventral funiculi of the spinal cord and was more prominent in the spinal cord than in the brain stem. Spongy change was not associated with axonal degeneration, inflammation, loss of myelin, or myelinolysis. Affected neurons, which were restricted to the brain stem and anterior horns of the spinal cord, varied in degree of vacuolization and often had nuclei that were displaced against the cell membrane (Fig. 4). Neuronal vacuolization was most prominent in the anterior horns of the cervical and lumbar intumescences. There was no neuron loss, gliosis, or perineuronal satellitosis at 30 days p.i. Early degenerative neuronal changes were central chromatolysis, peripheral localization of Nissl substance, and displacement of the nucleus against the cell membrane (Fig. 5A).

Virus replication in primary nerve cell cultures. To further establish that *ts*1 was able to infect and replicate more efficiently than wt MoMuLV-TB in the CNS, primary nerve cell cultures were infected as described above. Primary nerve cells were cultured in DMEM 10/10 under conditions which yielded mostly glial cells (Fig. 6A) or in Sf-Mod-DMEM, which promoted neuronlike cells and reduced the number of glial cells in the culture (Fig. 6B). The replication of wt and *ts*1 viruses in each of these cultures is shown in Table 2. In the case of the glial cell-rich cultures, *ts*1 replicated to an approximately 3× higher titer than that of the wt virus. However, in the neuron-rich culture the *ts*1 virus titer was ~500× that of the wt virus. This indicated that *ts*1 virus could infect and replicate more efficiently in the neuronlike cells than could wt MoMuLV-TB.

To establish that the virus being produced by the *ts*1-infected primary culture maintained its phenotypic characteristics, we examined the processing of $Pr80^{env}$ by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described above. The major glycoprotein precipitated by anti-gp70 from *ts*1-infected primary nerve cell cultures was Pr80 (Fig. 2), indicating that the precursor was not processed in these cultures. In contrast, the major protein band from wt-inoculated primary nerve cell cultures was gp70, with lesser amounts of $Pr80^{env}$ (Fig. 2), indicating normal processing of the precursor. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the processing of $Pr80^{env}$ was not done in primary nerve cell cultures grown in Sf-Mod-DMEM (to enrich for neurons) because of the low number of nerve cells per plate after selection procedures. These studies showed that not only was the *ts*1 phenotype retained in primary nerve cell cultures but all results with the primary nerve cell cultures were consistent with the in vivo findings.

DISCUSSION

Our studies showed that *ts*1 replicated in the CNS, especially in the spinal cord, much more efficiently than did the

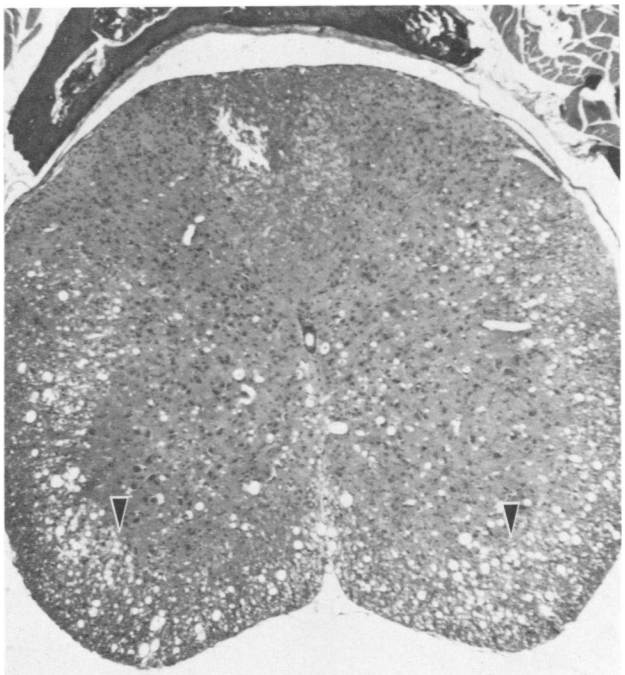

FIG. 3. Section of spinal cord from the cervical intumescence of a 30-day-old inbred CFW/D mouse infected with the *ts*1 mutant of MoMuLV-TB. Lateral and ventral funiculi (myelinated areas) had symmetrical spongy change most prominent at the gray matter-white matter interface (arrowheads). (H&E stain.) Magnification, ×12.5.

parental wt virus. This enhanced neurotropism of *ts*1 virions was further indicated by the observation that *ts*1 grew to a high titer in primary cultures of neurons of CFW/D mice, whereas wt virus showed barely detectable infectivity. The seeming paradox that *ts*1 virus replicates better than its wt counterpart in CNS tissues despite its temperature sensitivity and reduced ability to process $Pr80^{env}$ may be explained as follows. The inefficiency in processing the *env* precursor polyprotein to gp70 and p15E by *ts*1, which resulted in the accumulation of $Pr80^{env}$ in infected cells, is most pronounced at the restrictive temperature (38 to 39°C). However, the ratio of $pr80^{env}$ to gp70 is still relatively greater in *ts*1-infected cells than in wt-infected cells at the permissive temperature (34 to 36°C), indicating that at these temperatures *ts*1 is still somewhat defective in its ability to process $Pr80^{env}$ (24). The nonprocessing of $Pr80^{env}$ protein, however, does not inhibit the virus from being released from the infected cells. This phenomenon is also supported by the demonstration that murine sarcoma viruses which lack *env* proteins are released from infected cells (20). Moreover, at the permissive temperature the amount of infectious *ts*1 virus detected in the supernatant is comparable to that of wt virus, indicating that the slight reduction in the processing of $Pr80^{env}$ at this temperature probably has no effect on the infectivity of the *ts*1 virion. However, we do not know whether the slight accumulation of $Pr80^{env}$ protein at this temperature would have any effect on the nerve cells in vivo. The efficient replication of *ts*1 in infected mice despite its *ts* phenotype is consistent with the report that the body temperature of young mice (1 to 20 days old) maintained at room temperature is ~34°C and that the body temperature of adult mice (more than 3 weeks old) is ~38.4°C (5). It therefore appears that the replication of *ts*1 in young mice is not restricted and *ts*1 virus could spread to the CNS and establish infection in the nerve cells. After 20 days of age, as the mice achieve adulthood, their body temperature may become more restrictive to the ability of *ts*1 to process $Pr80^{env}$ to gp70 and p15E, which may affect the amount of infectious virus produced (i.e., affect its ability to spread from cell to cell). However, in mice older than 20 days, the ability of *ts*1 virus to spread from cell to cell may not be as critical to its ability to induce paralysis because most of the cells are probably infected before 20 days of age.

Our histopathologic studies of *ts*1-infected mice 25 to 30 days p.i. showed vacuolar degeneration of motor neurons much more severe than that of wt-infected mice. This histologic change in *ts*1-infected neurons was not observed in other (e.g., spleen) cells infected with *ts*1 or wt MoMuLV. Furthermore, our experiments with primary nerve cell culture showed that neuron-rich cell culture supports the replication of *ts*1 virus much better than it does that of wt MoMuLV. We do not know why neurons are selectively more susceptible to damage by *ts*1 virus than wt MoMuLV.

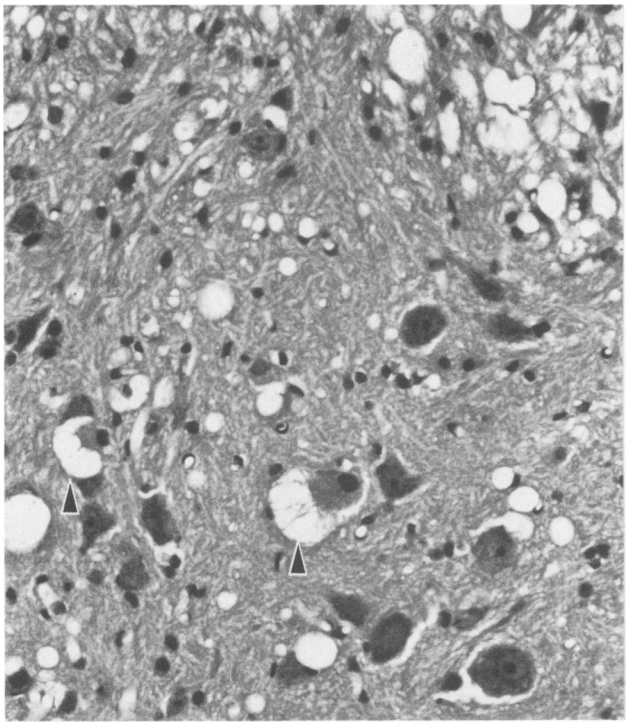

FIG. 4. Higher magnification of Fig. 3. Anterior-horn neurons contained one or more discrete or coalesced vacuoles of different sizes that often displaced nuclei against cell membrane (arrowheads). At 30 days p.i. with the *ts*1 mutant, there was no significant loss of neurons, gliosis, or perineuronal satellitosis. (H&E stain.) Magnification, ×1,128.

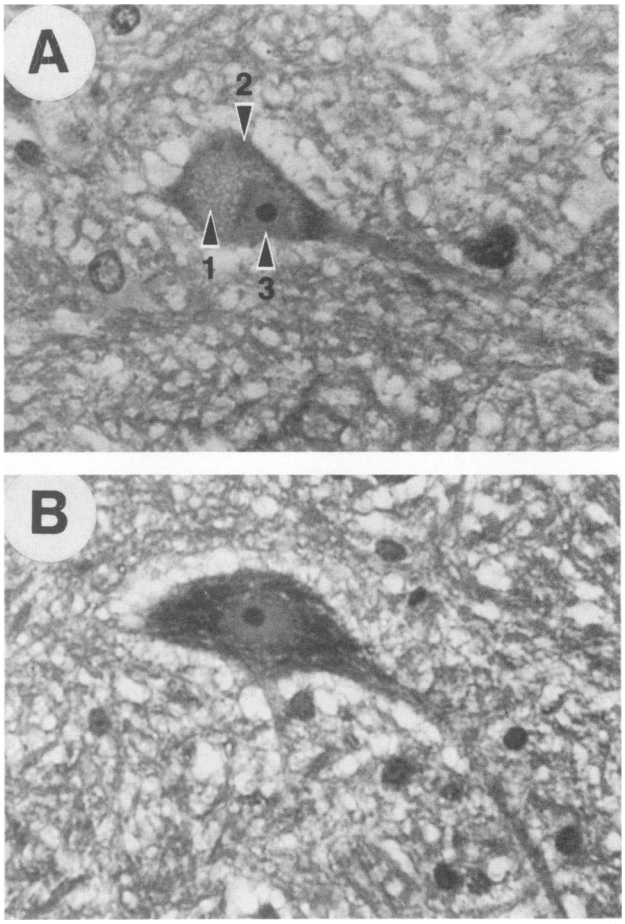

FIG. 5. (A) Early degenerative lesions in neurons of *ts*1-infected mice consisting of central chromatolysis (arrowhead 1), peripheral location of Nissl substance (arrowhead 2), and displacement of the nucleus against the cell membrane (arrowhead 3). (H&E stain.) Magnification, ×300. (B) Normal neuron with abundant evenly distributed Nissl substance and central nucleus with a single large nucleolus. (H&E stain.) Magnification, ×300.

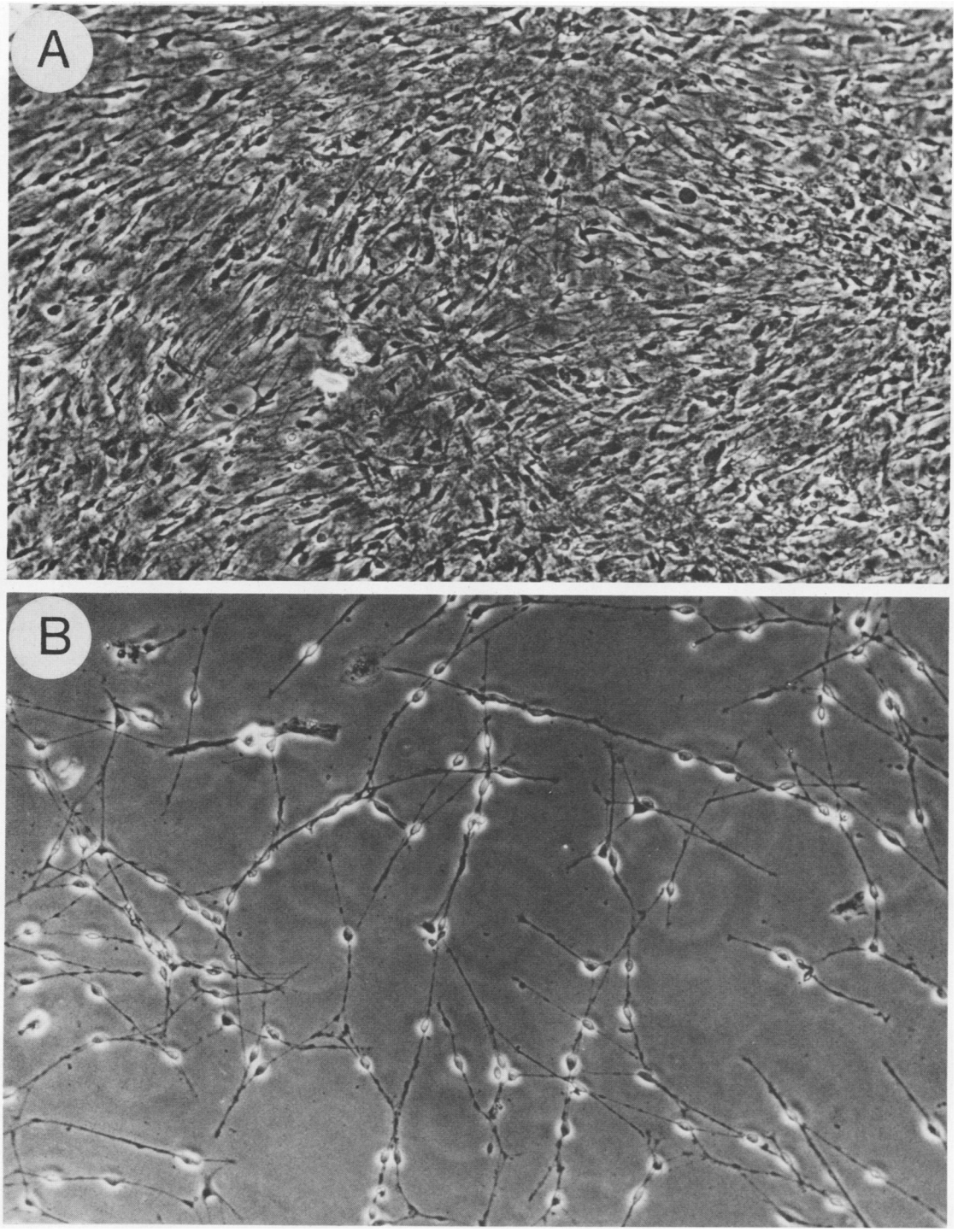

FIG. 6. (A) Primary nerve cell cuture in DMEM 10/10 consisting of mostly glial cells. Magnification, ×75. (B) Primary nerve cell cultures in Sf-Mod-DMEM consisting of mostly neuronlike cells. Magnification, ×75. Primary nerve cell cultures were prepared from fetal mouse spinal cords as described in the text.

Nor do we know why *ts*1 replicates better than wt MoMuLV in the CNS and primary neuron-rich culture. The similar although much less severe degeneration of neurons observed in wt MoMuLV-infected mice when compared with that of *ts*1-infected mice suggests that at least early in the development of neuronal lesions similar functional disturbances occur in *ts*1- and wt-infected mice. These disturbances, however, are somehow brought under control in wt-infected mice, because paralysis is never observed clinically. This may be a result of the enhanced ability of *ts*1 to gain entry into nerve cells so that all or most of the nerve cells are infected or to replicate in the nerve cells or both. It is also possible that the replication of wt virus is restricted at a different level. For example, *ts*1 and wt viruses may differ in their ability to integrate in nerve cell DNA, or change(s) in the *ts*1 long terminal repeat promoter or enhancer region

TABLE 2. Titers of *ts*1 and wt MoMuLV-TB in primary CNS cultures in DMEM 10/10 and Sf-Mod-DMEM[a]

Primary nerve cell culture	Virus titer (IU/ml) for	
	MoMuLV-TB	*ts*1
DMEM 10/10	4.0×10^4	1.2×10^5
Sf-Mod-DMEM	2.1×10^1	9.5×10^3

[a] Primary cell cultures were established as described in the text. The number of cells per plate in the DMEM 10/10 culture was estimated to be $\sim 10^5$ (predominantly glial cells). The number of cells per plate in the Sf-Mod-DMEM culture was estimated to be $\sim 10^3$ (predominantly neuronlike cells). Supernatants obtained from primary cell culture were assayed with 15F cells at 34°C. Titers are given as the average from two samples.

may render the replication of *ts*1 in the nerve cell much more efficient than that of wt virus. This could result in the exaggerated synthesis of the precursor *env* protein in *ts*1-infected cells. Alternatively, the failure to process $Pr80^{env}$ efficiently to gp70 and p15E, which resulted in reduced amounts of *env* proteins on the virion envelope of *ts*1, may allow *ts*1 virions to bypass blockage at the cell surface and gain entrance into the neurons. Experiments to resolve these possibilities are under way.

The finding that a retrovirus has an enhanced ability to replicate in the CNS is not without precedence. Besides the neurotropic Cas-Br-E strains of MuLV (14), human T-cell leukemia virus was recently shown to replicate actively in the brain of certain patients with acquired immune deficiency syndrome encephalopathy (18).

The histologic lesions observed in *ts*1-infected mice are similar to those reported in studies with the Cas-Br-E strain of MuLV (4, 14–16) and to those found with slow virus diseases such as kuru, scrapie, and Cruetzfeldt-Jakob disease (12). We observed marked pathologic changes in the upper and lower motor neurons of the brain stem and anterior horn of the spinal cord, respectively. The distribution of spongy change in myelinated areas of the lateral and ventral funiculi suggested involvement of tracts mediating intersegmental spinal reflexes (fasciculus proprious) and of descending motor tracts (vestibulospinal tracts). However, in *ts*1-infected mice at 30 days p.i., there was no significant hypertrophic astrogliosis, neuronal loss, or loss of myelin as reported in Cas-Br-E MuLV-infected CNS tissue (4, 14, 16). This difference may be the result of the differences between the studies in the ages of the lesions.

The cell populations affected in the CNS of *ts*1-inoculated mice appear to be primarily motor neurons and their associated oligodendroglial cells. The pattern of lesion development in *ts*1-infected mice suggested the possibility that virus spread from infected neurons to adjacent cell populations had occurred. Our preliminary transmission electron microscopic studies show the presence of C-type virus both in neurons and glial cells from spinal cord sections (manuscript in preparation). Other studies have also attributed the CNS disease induced by the wild mouse retrovirus to a primary infection of neurons and oligodendroglial cells (4, 14, 20, 21). Neuronal changes have been attributed to alterations of neuronal metabolism, a direct toxic effect of viral proteins on neurons, defective viral replication in neurons, or infection of supportive cells and disruption of normal neuronal maintenance (14, 21).

The paralytogenic *ts* mutants of MoMuLV have been shown to be inefficient in processing the *env* precursor polyprotein, with a resultant intracellular accumulation of this precursor. The gradual accumulation of the $Pr80^{env}$ protein may cause damage or malfunction of the nerve cell. Our recent findings that a 1.6-kilobase-pair *Hin*dIII-*Bam*HI subgenomic fragment in the genome, comprising the 3′ end of *pol* and the 5′ end of *env* sequences of *ts*1, is responsible for the defect of intracellular processing of $Pr80^{env}$ and is associated with the induction of paralysis in vivo (26) further support the hypothesis that the failure to process $Pr80^{env}$ plays a role in the induction of paralysis in infected mice.

Although the molecular mechanism of *ts*1-induced paralysis is not known, recent studies in our laboratory suggest that the cellular lesion relates to an abnormal association of $Pr80^{env}$ with elements of the cytoskeleton. We have evidence that transport and processing of MoMuLV $Pr80^{env}$ is regulated by elements of the cell cytoskeleton. Further, the $Pr80^{env}$ of the paralytogenic *ts*1 mutant is defective in its association with the cytoskeleton at 39°C, resulting in a buildup of the precursor on the cytoskeleton and a failure in transport and processing (unpublished data). Because the cytoskeleton plays a major role in regulating cell structure and function, it is conceivable that accumulation of $gPr80^{env}$ on the cytoskeleton of nerve cells could lead to the functional and structural CNS lesions observed in this study.

Detailed histologic, immunohistochemical, and ultrastructural studies are in progress in our laboratory to investigate the development of lesions, virus spread, the cell types infected by the virus, and the extent the CNS is affected as a result of *ts*1 virus infection. With the establishment of a primary nerve cell culture in our laboratory, a closer look at virus-nerve cell interactions with specific nerve cell populations can also be initiated.

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