Noninflammatory Spongiform Polioencephalomyelopathy Caused by a Neurotropic Temperature-Sensitive Mutant of Moloney Murine Leukemia Virus TB

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Newborn inbred CFW/D mice were inoculated intraperitoneally with ts1, a neutropic temperature-sensitive mutant of Moloney murine leukemia virus TB (MoMuLV-TB), with the parental wild type (wt) MoMuLV-TB, or with culture medium. A progressive symmetric hindlimb paresis that progressed to paralysis was observed in ts1-infected mice. Wt-infected mice and control mice had no neurologic signs. The severity and progression of neurologic signs correlated with the location, development, and progression of lesions. Lesions consisted of 1) neuronal and glial cell vacuolization in the brain and the anterior horn of the spinal cord, 2) spongiform change in the associated neuropil, 3) spongiform change in lateral and ventral funiculi, and 4) late fibrillary gliosis in the brainstem. There was no inflammation. Lesions were symmetric, increased in severity with time, and consistently arose at specific times in specific nuclei and areas of the brain and spinal cord. Similar, but less severe, histologic lesions were observed in corresponding areas of the central nervous system from wt-infected mice. Ultrastructurally, neuronal and glial cell vacuolization in ts1-infected mice at 31 days after inoculation was caused by dilatation of the endoplasmic reticulum and Golgi complex. Virions were observed in extremely low numbers predominantly in extracellular space and budding from membranes of neurons and glial cells. Virions were not observed in the endoplasmic reticulum or Golgi complex of neurons, nor were there cytoplasmic vacuoles that contained abnormal virions. (Am J Pathol 1986, 124:457–468)

TEMPERATURE-SENSITIVE (ts) mutants, ts1, ts7, and ts11, of Moloney murine leukemia virus (MoMuLV) grown in thymus–bone marrow (TB) cell culture cause paralysis and death in inbred strains of mice.[1,2] The parental wild-type (wt) MoMuLV-TB produces lymphoma in the same mouse strains. Inoculation of newborn CFW/D mice with ts1 or wt virus results in a rapid increase in the concentration of infectious virus in plasma and spleen by 10 days after inoculation.[3] The concentration of virus in brain and spinal cord of ts1-infected mice increases gradually and reaches a titer comparable to that in the spleen and exceeds that in the plasma by 25 to 30 days after inoculation. Virus titer is approximately 200 times greater in ts1-infected spinal cord and approximately 20 times greater in ts1-infected brain than in the same wt-infected tissues at this time.[3] Substantially more $Pr80^{env}$ polyprotein is present in the central nervous system of ts1-infected mice than in wt-infected mice.[3] Paralytogenic ts mutants do not process *env* precursor polyprotein $Pr80^{env}$ to gp70 and p15E efficiently.[2] Nonparalytogenic ts mutants, ts3 and ts10, process $Pr80^{env}$ to gp70 and p15E normally.[2] This defect in paralytogenic mutants results in the accumulation of $Pr80^{env}$ polyprotein in infected cells and the production of virions with reduced amounts of gp70 and p15E.[2] A 1.6-kilobase-pair *Hin*dIII-*Bam*Hi fragment in the ts1 mutant genome comprising the 3′ end of *pol* and the 5′ end of *env* is responsible for the inability of the ts1 mutant to process $Pr80^{env}$ intracellularly.[4] Replacement of this ts1 fragment with a homologous fragment from wt virus prevents paralysis in inoculated mice.

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Table 1—The Effects of ts1 Virus Titer on the Latent Period and Life Span of CFW/D Mice

Number of mice inoculated	Injected iu (IP)	Number affected*	Average age at onset	Average age at death	
				Affected	Not affected
20	$0-10^1$	2	3 months	5 months	>12 months
20	10^1-10^2	3	2.8 months	5 months	>12 months
20	10^2-10^3	3	3 months	5 months	>12 months
20	10^3-10^4	20	2.5 months	6 months	
20	10^4-10^5	20	45 days	2.5 months	
20	10^5-10^6	20	24 days	31 days	

* Affected, = the development of neurologic signs.
IP, intraperitoneal injection; iu, infectious units.

The purpose of this paper is to describe and compare the neurologic and histologic findings in newborn CFW/D mice inoculated with the ts1 mutant of MoMuLV-TB with those findings in wt MoMuLV-TB-infected mice and to report preliminary ultrastructural findings in ts1-infected mice 31 days after inoculation. These findings will be compared with other neurotropic retrovirus models and will be discussed on the basis of the current knowledge of the molecular biology of ts1-MoMuLV-TB.

Materials and Methods

CFW/D mice were housed in polycarbonate shoebox (35 × 22 × 13 cm) type cages with wire tops and were maintained in an environmentally controlled isolation room. Mice were provided food and tap water *ad libitum* and were observed twice daily. Litters of newborn mice and their dams were moved to separate but identical isolation facilities prior to virus inoculation.

Parental wt-MoMuLV-TB was isolated from a sarcoma from a MoMuLV- and murine sarcoma virus-infected BALB/c mouse.[5] It has been propagated in TB cell culture and has been recently single-virus/single-cell cloned with a multiplicity of infection (moi) of 0.01.[6,7] Detailed procedures for virus purification have been described.[7] Ts1 virus was a spontaneous mutant of the parental wt-MoMuLV, and its isolation and characterization have been described.[5,6] It has been propagated in TB cell culture and has been recently single-virus, single-cell cloned at a moi of 0.01. The TB cell line was derived from CFW/D mice, and its features have been described.[5,8] A modified 15F direct focus-forming assay (nontransformed, nonproducer, sarcoma-positive, leukemia-negative cell line) was used to determine virus titers no more than 2 weeks prior to inoculation.[9,10] In a preliminary experiment, serial dilutions of ts1 virus were used to determine the infectious units (iu) necessary to induce paralysis and to determine the latent period and time course of infection in a total of

Table 2—Criteria for Scoring Lesions

Score	Symmetry*	Brain lesion			Number of paired nuclei or areas affected	Number of neurons affected per nucleus or area
		Neuropil spongiosis	Glial cell hypertrophy	Fibrillary gliosis		
0	No lesion	–	–	–	0	0
1	Present	+	+	+	1	≥2
2	Present	+ +	+ +	+ +	2	≥2
3	Present	+ + +	+ + +	+ + +	3 to 5	≥2
4	Present	+ + + +	+ + + +	+ + + +	>5	≥2

Score	Symmetry*	Spinal cord lesion			
		Gray matter spongiosis	Glial cell hypertrophy	Number of neurons affected	White matter spongiosis
0	No lesion	–	–	0	–
1	Present	+	+	1 to 5	+
2	Present	+ +	+ +	6 to 10	+ +
3	Present	+ + +	+ + +	11 to 15	+ + +
4	Present	+ + + +	+ + + +	>15	+ + + +

–, no lesions; +, mild; + +, moderate; + + +, marked; + + + +, severe.
* Early or mild lesions were occasionally asymmetric.

120 newborn CFW/D mice (Table 1). Twenty mice per dilution were inoculated intraperitoneally at less than 24 hours of age with 100 µl of the respective dilution.

Neurologic signs were evaluated in three groups of 25 mice each, inoculated intraperitoneally at less than 24 hours of age with 10^5–10^6 iu of ts1 or wt-MoMuLV-TB. Control mice were inoculated with 100 µl of culture medium. Mice were evaluated weekly for change in body weight, righting reflex (mid-air and lateral recumbancy), withdrawal reflex (fore and hind limb), perineal reflex, muscle tone (fore and hindlimb), conscious pain response (superficial and deep pain), and subjective limb movement and control. Pain response was differentiated from withdrawal reflexes by observing the mice after a toe pinch for visual recognition of the stimulus, lip and facial movement or vocalization during the stimulus, and attempts to bite the stimulus.

Additional newborn CFW/D mice were randomly divided into ts1, wt, and control groups and were inoculated intraperitoneally at less than 24 hours of age with 10^5–10^6 iu of respective virus or with 100 µl of culture medium. At 7, 14, 21, 28, and 31 days after inoculation, 3 mice from each group were evaluated for neurologic signs. Mice from Days 7 and 14 were killed and the brain and spinal cord were immediately dissected and, along with other tissues, were fixed by immersion in phosphate-buffered (pH 7.2) 10% formalin. Mice from Days 21, 28, and 31 were anesthetized with phenobarbital and perfused through the aorta via the left ventricle with 50 U/ml of heparin in phosphate-buffered saline (pH 7.4, 330 mOsm, 37 C), followed by phosphate-buffered 10% formalin. Brain, spinal cord and remaining tissues were removed and immersed in phosphate-buffered 10% formalin. Tissues were slowly decalcified as necessary, dehydrated, embedded in paraffin, sectioned at 5 µ, and stained with hematoxylin and eosin (H&E). Identification of nuclei was based on established anatomic landmarks.[11] Lesions were scored for severity on the basis of the criteria listed in Table 2 and expressed as a mean score for mouse group and date of evaluation (Table 3). Brains and spinal cords were sectioned at approximately the levels indicated in Table 3.

Spinal cord from ts1-infected mice 31 days after inoculation was fixed for preliminary ultrastructural evaluation by the perfusion method described previously except for the use of phosphate-buffered (pH 7.4, 37 C) 2.5% glutaraldehyde as the fixative. Spinal cord was trimmed to 0.5-mm blocks that contained anterior horn or lateral/ventral funiculi, postfixed in osmi-

Table 3—Scoring of CNS Lesions: Sequential Development and Severity

			Level of CNS						
			Brain					Spinal cord	
Day pi	Group	n	A	B	C	D	E	F	G
7	co	3	0	0	0	0	0	0	0
	ts1	3	0	0	0	0	0	0	0
	wt	3	0	0	0	0	0	0	0
14	co	3	0	0	0	0	0	0	0
	ts1	3	0	1.0	1.7	2.0	3.0	1.7	1.0
	wt	3	0	0	0.3	0	2.5	1.0	0.7
21	co	3	0	0	0	0	0	0	0
	ts1	3	0.6	3.0	3.3	3.5	4.0	2.0	2.7
	wt	3	0	0.3	1.3	2.7	3.0	1.7	2.0
28	co	3	0	0	0	0	0	0	0
	ts1	3	0.3	2.0	3.0	3.5	3.7	2.0	2.7
	wt	2	0	0.5	1.5	2.5	2.0	2.0	2.0
31	co	3	0	0	0	0	0	0	0
	ts1	3	0	2.3	2.7	3.3	4.0	3.3	3.7
	wt	0	ND	ND	ND	ND	ND	ND	ND
42	co	3	0	0	0	0	0	0	
	ts1	0							
	wt	2	0	0	0	1.5	2.5	2	2
150	co	3	0	0	0	0	0	0	0
	ts1	0							
	wt	2	0	0	0	2	1	1	1

A, frontal cerebral cortex, olfactory nuclei; B, frontoparietal cerebral cortex, anterior corpus callosum; C, frontoparietal cerebral cortex, thalamic/hypothalamic nuclear groups; D, striate cerebral cortex, superior/inferior colliculi; E, cerebellar cortex-brainstem, cerebellar roof nuclei; F, cervicothoracic spinal cord enlargement; G, thoracolumbar spinal cord enlargement.

ND, not done; pi, postinoculation; co, control; ts1, ts1 virus; wt, wt virus; n, number of mice evaluated in each group.

um tetroxide, dehydrated, embedded in Epon, thick-sectioned, stained with toluidine blue for preliminary evaluation, subsequently thin-sectioned (silver interference), stained with uranyl acetate and lead citrate, and examined with a JEOL 100CX transmission electron microscope at 80 kv. Identification of ultrastructural features was based on established ultrastructural morphology of individual cells in the nervous system and on established relationships between cells within the neuropil.[12]

Results

Clinical Findings

Tsl-infected mice weighed 10% and 30% less than wt-infected or control mice at Days 21 and 24 after inoculation, respectively. Neurologic examinations were done at weekly intervals (Table 4). Mild generalized body tremors were first observed in tsl-infected mice at Day 21. By Day 26, tsl-infected mice were reluctant to move and body tremors were intensified by manipulation. Twenty-four hours later the mice developed symmetric hindlimb paresis, and a change in stance was observed. Both femorotibial joints were positioned more lateral and both tibial-tarsal joints were positioned more caudal than normal. By Day 28, tsl-infected mice were more paretic, had their hindlimbs in abduction, and had little hindlimb movement. The body weight of tsl-infected mice at this time was approximately half that of wt-infected or control mice. During the next 24 to 48 hours, hindlimb paralysis developed, the coxafemoral joints were adducted, and the tibial-tarsal joints were somewhat extended. Hindlimb movement was limited to the tibial-tarsal joint. Kyphosis and frequent intention tremors were observed at this time. Both forelimbs had intention tremors and altered neurologic responses (Table 4) but no paresis or paralysis. From this point, tsl-infected mice deteriorated rapidly. Tremors increased in severity and could be elicited with any disturbance. Mice had no interest in eating or drinking; however, all had food in their stomachs at necropsy. All tsl-infected mice died by 32 days after inoculation. Cyanotic mucous membranes, exophthalmia, and piloerection were observed during the last 12–16 hours of life. Gross lesions were not observed in tsl-infected mice at necropsy; however, the hindlimb musculature appeared pale when compared with that of wt-infected and control mice. Abnormal neurologic responses were not observed in either wt-infected or control mice during the same experimental period.

Histologic Findings

Brain and spinal cord from mice inoculated with tsl-MoMuLV-TB had a progressive symmetric noninflammatory spongiform polioencephalomyelopathy. Lesions arose consistently at specific times in specific nuclei and areas of the brain and in the anterior horns of the spinal cord. In general, lumbar spinal cord lesions were more pronounced than cervical spinal cord lesions, and brainstem-cerebellar lesions were more pronounced than spinal cord lesions. Cerebellar nuclear lesion were more prominent than brainstem nuclear lesions early, but brainstem nuclear involvement became greater with time.

There were no lesions in the central nervous system of mice 7 days after inoculation with tsl virus (Table 3). By 14 days, mild symmetric lesions were observed in Lamina VI of the frontoparietal cerebral cortex, thalamic nuclei, nuclei of the reticular formation, lateral cerebellar nuclei, interpositus cerebellar nuclei, and gray matter of anterior horn of the spinal cord (Table 5). Involvement of cerebellar nuclei was more prominent

Table 4—Neurologic Evaluation of Tsl-Inoculated CFW/D Mice*

Age (Day pi)	Righting reflex		Withdrawal reflex					Muscle tone			
	Drop	Lat	RF	LF	RH	LH	Perineal reflex	RF	LF	RH	LH
7	ND	ND	3	3	3	3	+	3	3	3	3
14	3	3	3	3	3	3	+	3	3	3	3
21	2	3	3	3	2	2	+	3	3	2	2
31	1	1	2	2	1	1	+/−	2	2	1	1

	Superficial pain				Deep pain			
Day pi	RF	LF	RH	LH	RF	LF	RH	LH
7	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+
31	+	+	−	−	+	+	−	−

* 3, normal response; 2, moderately altered response; 1, markedly altered response; +, present; −, absent.
pi, postinoculation; drop, dropping reflex; lat, lateral; RF, right forelimb; LF, left forelimb; RH, right hindlimb; LH, left hindlimb; ND, not done.

Table 5—Brain Nuclei/Areas Affected and Relative Frequency of Occurrence

Group	Brain level*	Nuclei/area affected	
		Frequently	Less frequently
ts1	A	None	Neurons (frontal cortex)
wt	A	None	None
ts1	B	Lamina VI (cc) Medial Septal nucleus Nuclei of Broca	Lamina IV, V (cc)
wt	B	Lamina VI (cc)	None
ts1	C	Lamina VI (cc) Thalamic Nuclei	Lamina IV, V (cc) Hypothalamic nuclei Habenular nuclei Geniculate nuclei Premammilary nuclei
wt	C	Lamina VI (cc) Thalamic nuclei	Hypothalamic nuclei
ts1	D	Deep mesencephalic nucleus Red nucleus Inferior colliculus Nuclei of lateral lemniscus	Superior Colliculus Pontine nucleus Pedunculopontine nucleus
wt	D	None	Red nucleus Inferior colliculus Nuclei of lateral lemniscus Deep mesencephalic nucleus Pontine nucleus Pedunculopontine nucleus
ts1	E	Lateral cerebellar nucleus Interpositus cerebellar nucleus Reticular formation nuclei Vestibular nuclei Pontine reticular nuclei Cochlear nuclei Trapezoid nuclei Nucleus, spinal tract, 5th nerve	Facial nucleus Olive nuclei Motor nucleus, 5th nerve
wt	E	Lateral cerebellar nucleus Interpositus cerebellar nucleus Reticular formation nuclei Vestibular nuclei Cochlear nuclei	Pontine reticular nucleus Facial nucleus Nucleus, spinal tract, 5th nerve Trapezoid nuclei Olive nuclei

* Refer to Table 3 for brain levels.
cc, cerebral cortex.

at this time, when compared with brainstem nuclear involvement. Other brain nuclei and areas were affected less frequently (Table 5). Neurons and glial cells had small, clear cytoplasmic vacuoles with distinct borders that, in general, did not displace the nucleus or were swollen and had granular cytoplasm. Similar vacuoles were present in the neuropil associated with groups of affected neurons and glial cells. By Day 21 after inoculation with ts1 virus, brain involvement, in addition to that described for Day 14, included the vestibular nuclei, red nuclei, and additional nuclei of the reticular formation (Table 5). Other nuclei were affected less frequently (Table 5). Brainstem nuclear lesions were greater in extent and severity than cerebellar nuclear lesions at this time. Lesions in Lamina VI of the frontoparietal cortex were mild, and Lamina V and Lamina IV were involved occasionally.

Symmetric spongiform change in the neuropil of the brain and spinal cord increased in severity from Day 21 through Day 31 after inoculation (Figure 1A and B). Neurons had severe chromatolysis, and cytoplasm contained one or more discrete or coalesced clear vacuoles with distinct borders or was coarsely reticulated (Figure 2A and B). Nuclei in vacuolated neurons were displaced against cell membranes and had condensed chromatin and dense nucleoli; in less affected neurons, clear perinuclear halos were observed (Figure 2A and B). Axons contained or were surrounded by clear vacuoles that demarcated their path through the neuropil of the brainstem. There was no apparent loss of neurons; however, there were occasional necrotic neurons with shrunken acidophilic cytoplasmic and pyknotic nuclei. There were numerous swollen glial cells with abundant granular or vacuolated cytoplasm (Figure 2A and B).

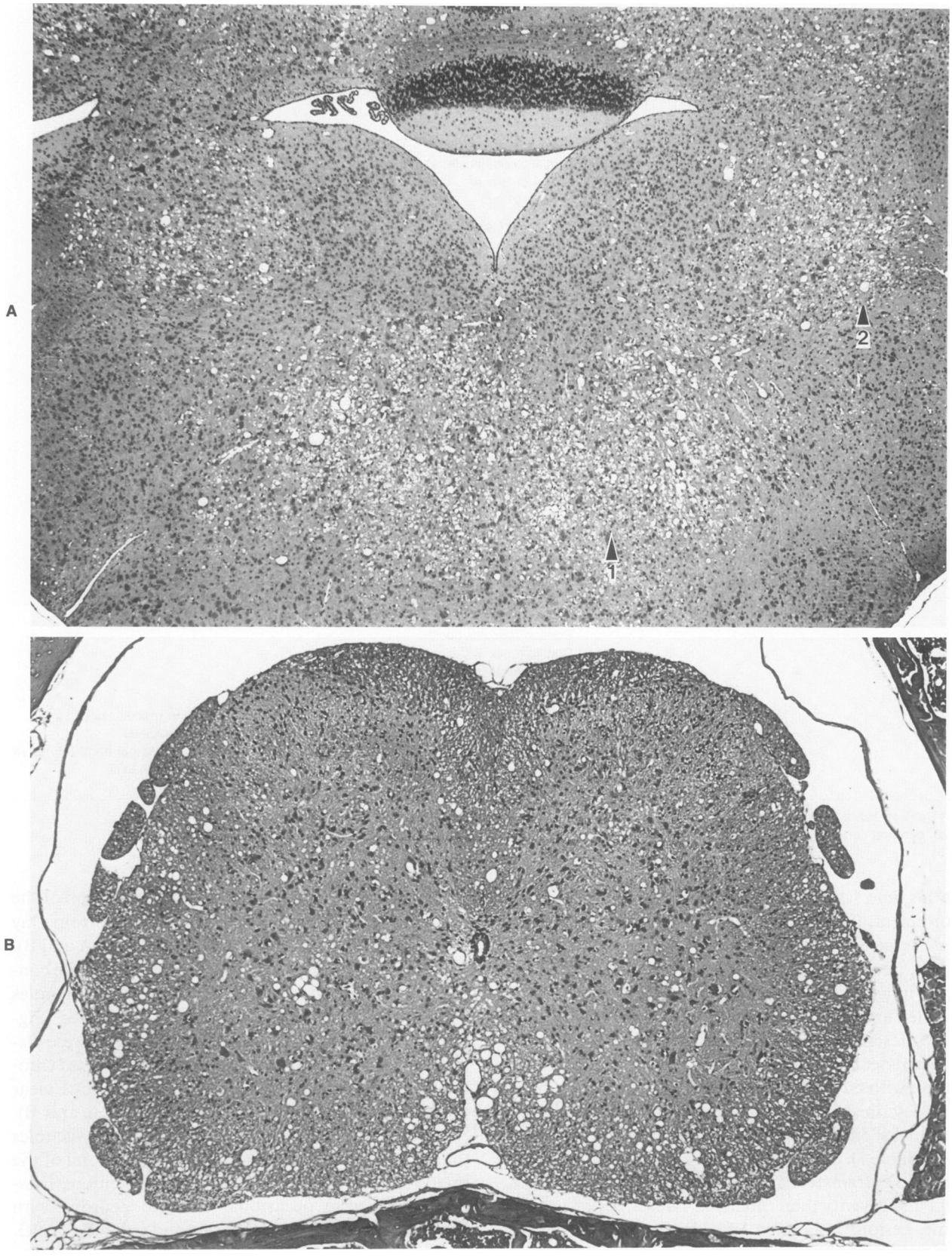

Figure 1—Central nervous system from a CFW/D mouse 31 days after inoculation with ts1-MoMuLV-TB. **A**—Transverse section of brainstem including gigantocellular reticular nucleus (*1*) and vestibular nucleus (*2*). Symmetric spongiform change that involved nuclei and the associated neuropil. (H&E, ×38) **B**—Transverse section of thoracolumbar spinal cord enlargement. Spongiform change is present in anterior horn gray matter and in lateral and ventral funiculi. (H&E, ×48)

Fibrillary astrogliosis was evident at Day 31 after inoculation in brainstem (Figure 2A), but a significant fibrillary gliosis was not observed in anterior horns of the spinal cord (Figure 2B).

Lateral and ventral funiculi had generalized symmetric spongiform changes that in some instances were most pronounced at the white matter/gray matter interface (Figure 1B). There were no swollen axons in these areas. This lesion increased in severity in ts1-MoMuLV-TB-infected mice from Day 14 through Day 31 after inoculation (Table 3). A similar, mild spongiform change was observed in anterior nerve roots of spinal nerves arising in the cervical and lumbar enlargements but was not present in distal segments. There were no lesions in posterior nerve roots, ganglia, and gray horns, or in the cuneate and gracile fasciculi.

Similar but less severe lesions were observed in the same nuclei and areas of the brain and spinal cord of mice inoculated with wt virus as were described for ts1-infected mice (Tables 3 and 5). These lesions were maximal at Day 21 after inoculation and then slowly decreased in severity by Day 42. Lesions, although mild, were still observed in the central nervous system of wt-infected mice at 150 days after inoculation.

There were no lesions in the central nervous system of control mice and no lesions in lung, kidney, liver, myocardium, thymus, spleen, and intestine in any group. Striated muscle of the hind limbs from mice 31 days after inoculation with ts1 virus was mildly atrophic.

Preliminary Ultrastructural Findings

Anterior horn from the cervicothoracic spinal cord enlargement from mice 31 days after inoculation with ts1 virus revealed a continuum of lesions affecting neurons and glial cells. Lower motor neurons with vacuolated or reticulated cytoplasm had severe dilatation of the rough and smooth endoplasmic reticulum and the Golgi complex and condensation of nuclear and nucleolar chromatin (Figure 3A). Organelles were confined in narrow bands of cytosol by these dilated membrane-bound spaces (Figure 3B). Dilatation of the membrane systems extended into axons and dendrites. Early neuronal

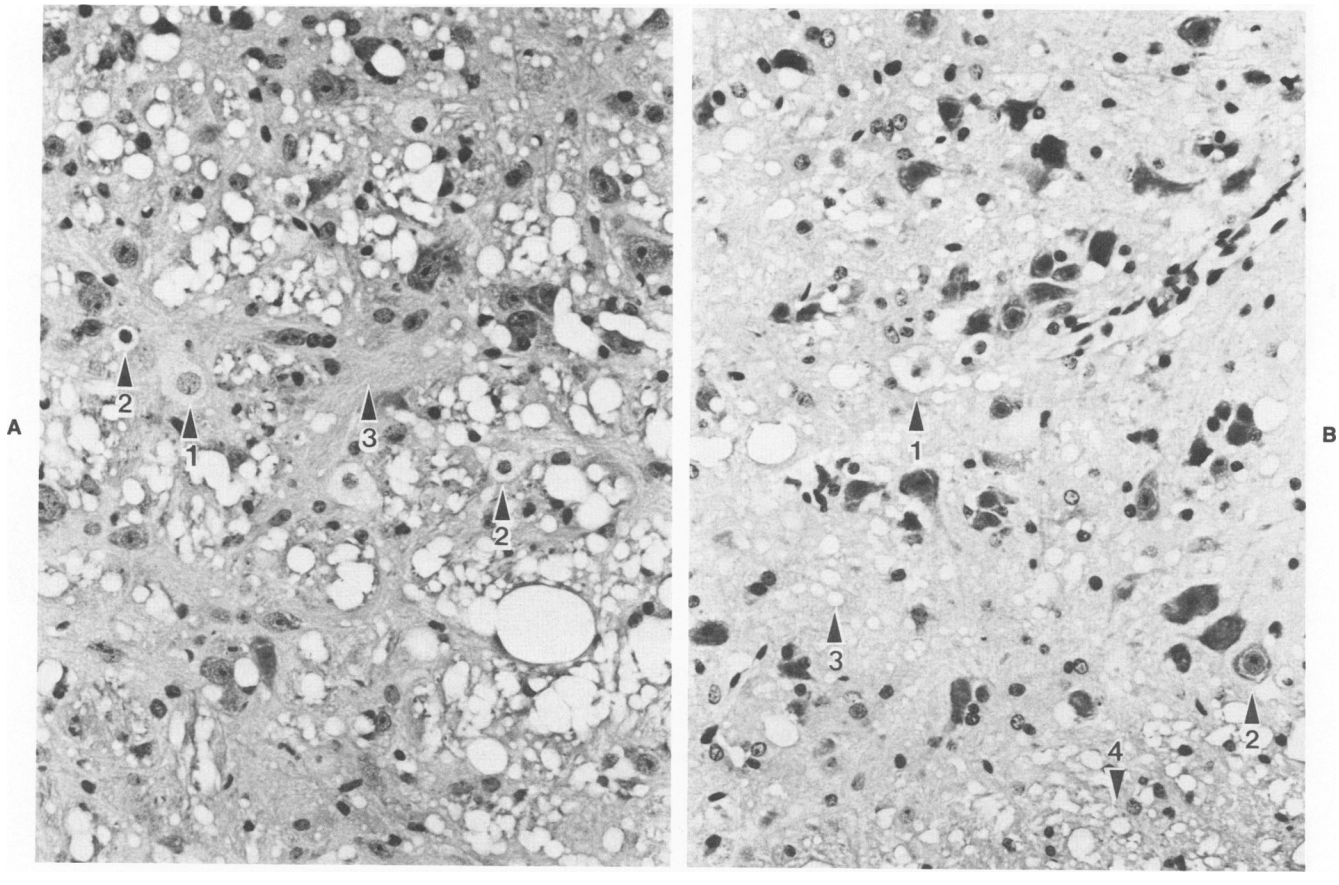

Figure 2—CNS from a CFW/D mouse 31 days after inoculation with ts1-MoMuLV-TB. **A**—Transverse section of a gigantocellular reticular nucleus and its associated neuropil. Swollen neuronal cell body (*1*), hypertrophied glial cells (*2*), and fibrillary gliosis (*3*) are admixed with extensive neuropil spongiform change. (H&E, ×229) **B**—Transverse section of anterior horn at thoracolumbar enlargement. Vacuolated and coarsely reticulated cytoplasm of the cell body of a neuron (*1*), perinuclear neuronal halo (*2*), anterior horn neuropil spongiform change (*3*), and spongiform change in a white matter funiculus (*4*). (H&E, ×250)

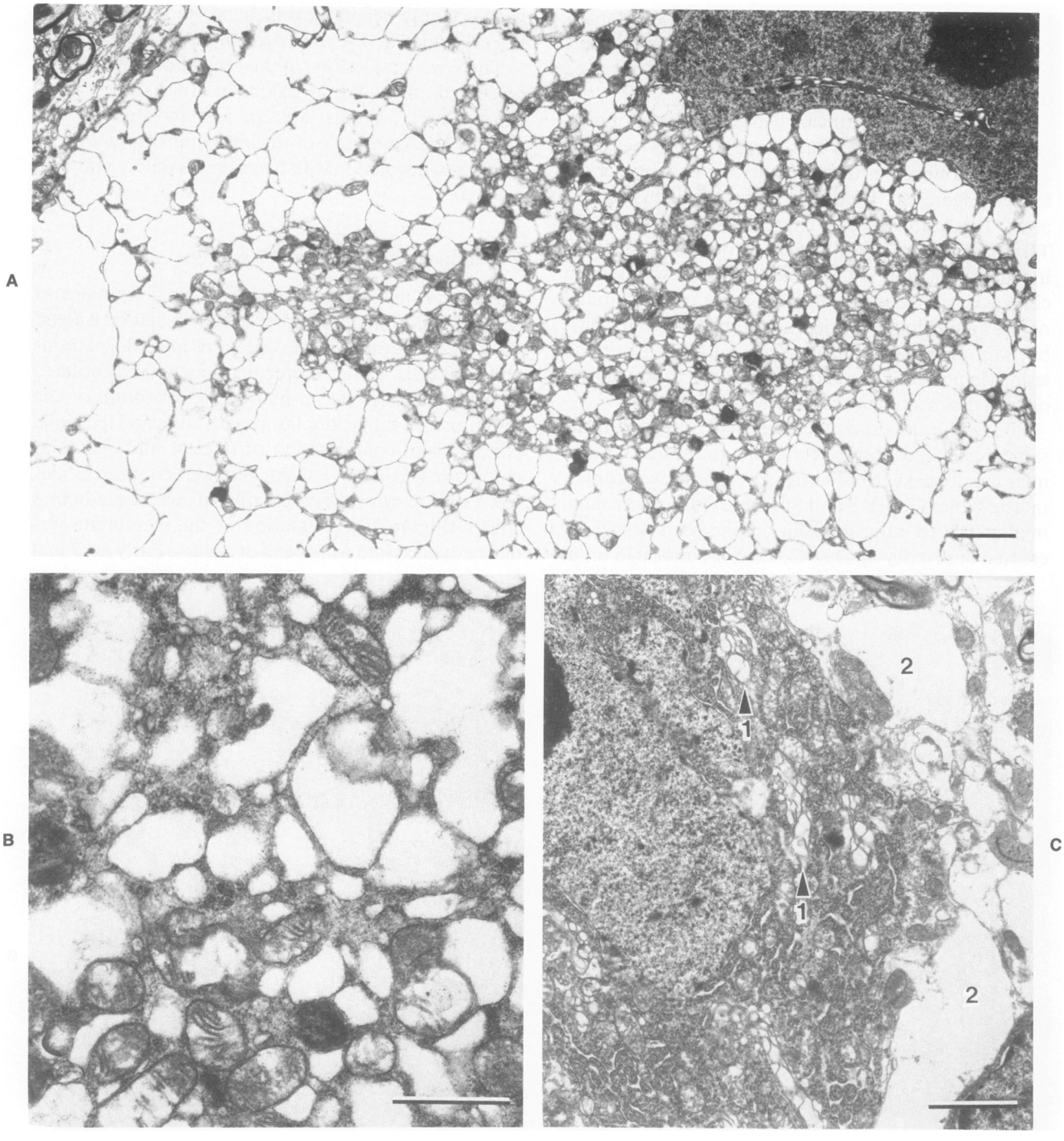

Figure 3—Transmission electron micrographs from the cervicothoracic spinal cord enlargement of a CFW/D mouse 31 days after inoculation with ts1 MoMuLV-TB. **A**—The endoplasmic reticulum and Golgi complex are dilated and contain a poorly electron-dense flocculent material. Organelles are confined in scant cytosol; and nuclear and nucleolar chromatin is condensed. Bar = 2.0 μ. (Uranyl acetate and lead citrate, ×5500) **B**—Dilated membrane system and organelles confined in scant cytosol. Bar = 1.0 μ. (Uranyl acetate and lead citrate, ×18,900) **C**—Moderately dilated and whorled Golgi complex (*1*) and dilated cell processes (*2*) adjacent to a neuron. Bar = 2.0 μ. (Uranyl acetate and lead citrate, ×7100)

lesions were abnormal whorling and dilatation of the Golgi complex (Figure 3C).

Glial cells had edematous cytosol affecting cell bodies and processes, mildly dilated endoplasmic reticulum and Golgi complex, disorganized organelles, and haphazardly arranged intermediate filaments. Virus budding sites and immature and mature virus particles were observed in extremely low numbers predominantly in association with plasmalemma of neuronal and glial processes and in extracellular spaces (Figure 4). Virus

particles were surrounded by an outer envelope derived from host cell membrane. Immature virus particles had a nucleoid with a spherical or hexagonal electron-lucent center; whereas virions had granular spherical or hexagonal electron-dense nucleoids. Cylindrical virus particles were not observed in the central nervous system (CNS). Virions could not be identified within the endoplasmic reticulum or Golgi complex of neurons or neuronal processes. There were no membrane-bound cytoplasmic vacuoles that contained abnormal viral particles in neurons.

Discussion

A noninflammatory spongiform polioencephalomyelopathy was produced in newborn CFW/D mice inoculated with cloned ts1-MoMuLV grown in TB cell culture. Neurologic findings of progressive hindlimb paresis, ataxia, and tremors followed by generalized tremors and flaccid hindlimb paralysis suggested a lower motor neuron disorder. Gross and histologic lesions in striated muscle were also suggestive of early neurogenic atrophy compatible with lower motor neuron disease. These findings were similar to those reported in previous studies with murine neurotropic retroviruses.[14]

Histologic lesions were similar to those reported in the wild mouse virus (Cas-Br-M, WM1504 E, WM1504 M) models[14-17] and for the slow virus diseases of scrapie, kuru, and Creutzfeldt-Jakob disease.[18] Our findings indicated involvement of the anterior horns of the cervical and, most prominently, lumbar spinal cord enlargements and lateral and ventral funiculi. Lesions appeared in cervical and lumbar spinal cord simultaneously; however, the reason for the greater severity in the lumbar spinal cord was unknown. This difference in severity explained the dominance of hindlimb involvement in neurologic evaluations. Histologic findings confirmed the neurologic signs of flaccid paralysis caused by lower motor neuron disease. Lesions in the lateral and ventral funiculi were diagnosed as symmetric spongiform change. This distribution suggested major involvement of upper motor neurons of the extrapyramidal system. It was thought that neurologic signs of upper motor neuron disease were not observed in ts1-infected mice because they were masked by a combination of lower motor neuron disease and cerebellar nuclear lesions.

Spongiosis of the fasciculus proprius was observed adjacent to funiculi with spongiosis. This lesion suggested there was dysfunction of neurons integrating intrasegmental and intersegmental reflexes. There were no

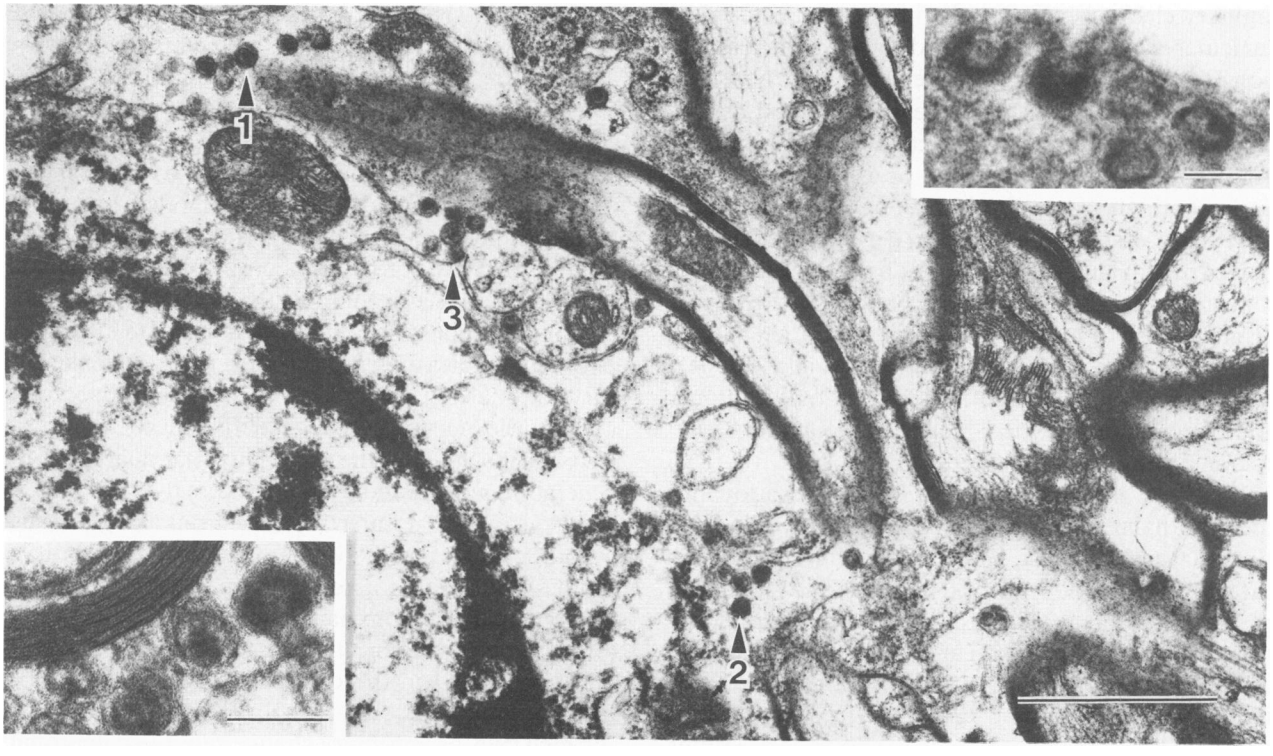

Figure 4—Transmission electron micrographs of the cervicothoracic spinal cord enlargement of a CFW/D mouse 31 days after inoculation with Ts1-MoMuLV-TB. Virus particles in extracellular space (*1*), budding from a glial cell plasmalemma (*2*), and budding from a dendrite or preterminal unmyelinated axon (*3*). Bar = 1.0 μ. (Uranyl acetate and lead citrate, ×25,900) **Upper right inset** shows the morphologic features of membrane budding sites from a neural cell plasmalemma. Bar = 0.1 μ (Uranyl acetate and lead citrate, ×136,000) **Lower left inset** shows virus budding from plasmalemma of a neural cell and virions free in the extracellular space. Bar = 0.1 μ (Uranyl acetate and lead citrate, ×102,000)

lesions in dorsal funiculi; but because of overlap between the ascending spinothalamic tract and descending corticospinal and rubrospinal tracts, involvement of this sensory pathway could not be precisely evaluated histologically.[13] Reduced pain responses indicated involvement of ascending sensory tracts. These findings were thought to be due to lesions in thalamic nuclei, which function as the major sensory correlation center in animals where the extrapyramidal system dominates motor functions.[13]

Lesions were observed in nuclei of the extrapyramidal system (red, vestibular, and gigantocellular reticular nuclei), nuclei of the brainstem with integrative or associative motor functions (reticular formation, cerebellar, thalamic nuclei), nuclei with major sensory functions (thalamic nuclei, superior and inferior colliculi), and nuclei of cranial nerves.[11,13] It is not known why, in general, cerebellar nuclear lesions were more prominent early after inoculation than were brainstem nuclear lesions, why brainstem nuclear lesions dominated all other areas of the CNS later after inoculation, or why brainstem-cerebellar lesions were greater in severity than spinal cord lesions. These findings may be related to viral tropism or to the susceptibility of specific neuron populations to virus-induced injury. Degeneration of extrapyramidal neurons was probably in part responsible for spongiosis of lateral and ventral funiculi. Preliminary electron-microscopic findings indicated that funicular spongiosis was caused by granular dissolution of the axoplasm and by myelin loss due to separation of myelin lamellas. Whether myelin loss was secondary to axonal degeneration, caused by viral-induced injury to oligodendroglial cells, or due to a combination of both mechanisms could not be determined from preliminary evaluations. Wallerian degeneration, separation of myelin lamellas, redundant myelin sheaths, neuronal and oligodendrocyte degeneration, and granular disintegration of axoplasm have been reported in electron-microscopic studies with Cas-Br-M and W1504E retroviruses.[14,17,19] These authors felt that both neuronal and oligodendrocyte viral-induced degeneration were responsible for these changes.[14,17,19]

Lesions in the nuclei of the reticular formation and the hypothalamus were extensive and severe. It is plausible that lesions in these nuclei may have ultimately led to the death of tsl-infected mice due to dysfunction of respiratory, consciousness, or vasomotor centers. Lesions in the thalamic nuclei and the rostral and caudal colliculi as well as the loss of pain responses indicated sensory involvement and explained the apparent involvement of sensory systems in the absence of lesions in posterior gray horn and dorsal funiculi of the spinal cord. These findings are in contrast to the apparent lack of sensory system involvement in other models.[14-17]

It is not clear why lesions would favor localization in extrapyramidal nuclei over pyramidal neurons. This may be a reflection of the importance of the former system in motor function in laboratory animals.[13] Integration of motor systems with sensory systems may explain the spread of lesions to sensory nuclei, but it does not account for the specificity of nuclei involved. This specificity may be regulated in part by surface receptors for virions that are common between neuronal and glial cell populations.[15,20] It is plausible, however, that specificity is determined by endothelial cell surface receptors and the distribution of neural and glial cell lesions is a reflection of this specificity. In addition, endothelial cells and glial cells of the brain may possibly serve as important sites for viral integration and replication in a dividing-cell population, in contrast to the postmitotic status of neurons. Although at this time we have no evidence to suggest how virus spreads to the CNS after intraperitoneal inoculation, others using wild mouse murine leukemia virus have demonstrated that after hematogenous spread, virus infects endothelial cells and spreads contiguously into the neuropil by budding from the abluminal surface of the endothelium through the basement membrane.[19] Infection of specific glial and neural cells apparently follows shortly thereafter.

Preliminary electron-microscopic evaluation demonstrated viral particles in extracellular space, associated with the Golgi complex and plasmalemma of glial cells and budding from neuronal plasmalemma. Degenerative changes observed in neurons and glial cells suggested that virus infection caused the cellular changes observed in neurons and glial cells. This conclusion was in agreement with findings in other models.[14-17] Caution must be used in this interpretation in our studies because, in contrast to other neurotropic retrovirus systems, virus was never found in the endoplasmic reticulum or Golgi complex or budding aberrantly into cytoplasmic vacuoles of neurons. The intimate association between glial cells, especially astrocytes, and neurons and the role of astrocytes in regulating the neuronal environment suggested the possibility that neuronal degeneration was secondary to astrocyte dysfunction. It was likely that virus infection of neurons and glial cells affected cellular metabolism, most significantly protein synthesis, necessary for normal membrane function and ion transport. Neurons had the most pronounced dilatation of endoplasmic reticulum and Golgi complex of all cells studied in the central nervous system. This change could be secondary to dysfunction of supporting glial cells or a primary change caused by neuronal infection with tsl virus.

We believe the latter hypothesis is most likely because experimental and spontaneous diseases with severe dys-

function of astrocytes are, in general, not associated with dilatation of neuronal endoplasmic reticulum or Golgi complex.[21,22] This dilatation could be explained by three possible mechanisms: 1) accumulation of material in the endoplasmic reticulum or Golgi complex, 2) excessive formation of membrane due to massive insertion or defective processing of proteins in the membranes, and 3) alteration of cytoskeleton that results in abnormal axoplasmic transport. Similar mechanisms may occur in glial cells. Ts1 virus is defective in converting $Pr80^{env}$ to gp70 and p15E, and it is plausible that accumulation of *env* protein caused dilatation of the endoplasmic reticulum and Golgi complex. Electron-microscopy observation, however, suggests that there is no significant quantity of material within these membrane-bound spaces. $Pr80^{env}$ may be inserted into the membrane of the endoplasmic reticulum and Golgi complex or improperly processed after insertion, resulting in excessive formation of membrane. Lack of processing may also explain why such low numbers of virus particles are seen in neurons and glial cells. However, the inability of ts1 virus to process precursor $Pr80^{env}$ protein apparently does not restrict virus from budding and infecting adjacent cells. It has also been demonstrated (Soong, Wong, Basgall, Tompkins, unpublished data) that $Pr80^{env}$ binds to macrophage cytoskeleton; and if a similar mechanism occurs in neurons, this interaction may influence axoplasmic transport. Further studies are needed to define the role of $Pr80^{env}$ in the dilatation of the neuronal and glial endoplasmic reticulum and Golgi complex and its influence on cell degeneration. Neuronal degeneration in our system is a slow process that results in a progressive cumulative dysfunction of neurons. This injury is apparently irreversible and ultimately results in neuronal death and loss. Work with other murine neurotropic retrovirus models has produced hypotheses that suggest neuronal injury might be due to viral-derived proteins toxic to neurons,[23] to alterations in cyclic nucleotide metabolism,[24] or to interference with normal cell metabolism.[16]

Similar histologic neuronal and glial lesions were observed in CFW/D mice infected with wt virus. Lesions were not as severe as those in ts1-infected mice and regressed as the study progressed. There was no apparent loss of neurons. Wt virus does synthesize $Pr80^{env}$; however, it is converted to gp70 and p15E. It is possible that early in infection with wt virus enough $Pr80^{env}$ is synthesized to cause histologic changes without functional abnormalities that result in neurologic signs. Wt-infected mice appear able to resolve the injury by converting $Pr80^{env}$ to gp70 and p15E and possibly minimize the hypothesized dysfunction of cellular organelles or cytoskeleton associated with excessive early synthesis of $Pr80^{env}$. The difference in the progression of lesions between wt and ts1 viruses may also be explained by the fact that wt virus replicates less efficiently and produces less $Pr80^{env}$ in the CNS than does ts1 virus.[3]

The possibility exists that lesions in wt-inoculated mice were caused by contamination of inocula with ts1 virus or a spontaneous mutant. The former hypothesis has been essentially eliminated by repeated single-cell/single-virus cloning at an moi of 0.01.[5-7] In addition, our study demonstrated that all mice inoculated with as few as 10^3 iu of ts1 virus developed neurologic signs and died (Table 1). Unpublished data from our laboratory have shown that mice inoculated with mixtures of varied concentrations of ts1 and wt virus always developed neurologic signs. If lesions in wt-virus-inoculated mice were caused by ts1 virus contamination, then we would have expected these mice to have neurologic signs and die. We have never observed neurologic signs in any of our studies with wt-MoMuLV-TB. It is possible that a spontaneous mutant arose in our wt virus inoculum after purification; however, it is unlikely that an identical mutation would occur in all wt virus inocula. Molecular cloning of wt and ts1 viruses would provide purified virus inocula but probably would be no better than single-cell/single-virus cloning in TB cell culture. Molecular cloning would not eliminate the possibility of a postcloning spontaneous mutant, but the chances of identical mutations in separate clones are slight.

Clinical, histologic, and preliminary ultrastructural findings in our study are similar to those murine neurotropic retrovirus models using WM1504 or Cas-Br-M strains.[14-17] Our incubation (latency) period (26 days), time until death (32 days), and incidence of paralysis (100%) following intraperitoneal inoculation are shorter than in those models except when concentrated virus inocula were injected intracerebrally.[25] Clinical signs and neurologic evaluations are similar; however, the early appearance of signs in our system is a function of the shorter incubation period. Although motor neurons are affected predominantly, there is neurologic and histologic evidence for some sensory dysfunction in our system, which is in contrast to the other neurotropic retrovirus systems. The development and appearance of lesions in the neuropil are similar to the WM1504- and Cas-Br-M-induced lesions.[14-17] The ts1 virus system appears to have greater involvement of brainstem nuclei when compared with experimental disease induced by wild mouse neurotropic retrovirus.[15] Ultrastructurally, lesions in the ts1 virus system are very similar in most respects to other retrovirus models.[14-17] We never found large numbers of virus particles, abnormal virus particles, or virions that budded aberrantly into vacuoles in neurons, as have been reported in other studies with neurotropic murine retrovirus.[14,17,19] This suggests that there may be related but different mecha-

nisms causing neuronal degeneration in these various retrovirus systems. We believe that Pr80^{env} protein plays a crucial role in neuronal degeneration in tsl-infected mice. The polypeptides gp70 and p30 have been shown necessary to produce paralysis in systems that use the wild mouse virus.[26]

Tsl-MoMuLV-TB appears to be a unique model for study of the pathogenesis of motor neuron disease and associated virus-cell interactions. Genetic recombinations between specific subgenomic fragments of the parental wt-MoMuLV-TB and tsl-virus should allow for future studies of gene products responsible for paralysis.[4] This model may also be useful in future studies of mechanisms of motor neuron diseases such as amyotrophic lateral sclerosis and the human retrovirus (HTLV III) associated neuronal degeneration in some patients with acquired immune deficiency syndrome.

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