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# **Vacuolar Neuronal Degeneration in the Ventral Horns of SCID Mice in Naturally Occurring Theiler's Encephalomyelitis**

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## **Summary**

During a spontaneous outbreak of Theiler's encephalomyelitis severe combined immunodeficient mice developed high morbidity and high mortality. Histological lesions were localized in the ventral horns of the spinal cord and brain stem. The salient features were the severe vacuolar degeneration of neurones and glial cells and the absence of inflammatory cellular infiltrates. The clinical and pathological features of this outbreak indicate that the SCID mouse would be a much improved model for studying the mechanism of poliovirus infection and of virus-induced demyelinating diseases.

## **Introduction**

During the early 1930s, Theiler reported the isolation of a virus from the central nervous system (CNS) of mice that had developed spontaneous flaccid paralysis of the limbs (Theiler, 1934, 1937). Since then, there have been few reports of natural disease caused by Theiler's mouse encephalomyelitis virus (TMEV) (Theiler and Gard, 1940a; Thompson, Harrison and Meyers, 1951; McConnel, Huxsoll, Garner, Spertzel, Warner and Yager, 1964). Natural infection results in replication of TMEV in the gastrointestinal mucosa (Olitsky, 1939; Dal Canto and Lipton, 1977; Zurbriggen and Fujinami, 1988). Rarely (0.01 per cent), this virus infects the CNS where it causes encephalitis resulting in flaccid paralysis of the hind limbs; front legs are sometimes affected. This is the only clinical sign likely to appear during the course of natural infection. (Lipton and Rozhon, 1986; Jacoby, 1988; Anonymous, 1991).

Present knowledge of the effects of TMEV infection has come mostly from studies of experimental infections with this virus (Theiler and Gard, 1940a and b; Lipton and Dal Canto, 1976a, 1979; Dal Canto and Lipton, 1977; Penney and Wolinsky, 1979; Lorch, Friedmann, Lipton and Kotler, 1981; Rosenthal, Fujinami and Lampert, 1986; Brownstein, Bhatt, Ardito, Paturzo and Johnson, 1989). Intracerebral infection (IC) of immunocompetent adult mice with TMEV produces a characteristic biphasic CNS disease (Lipton, 1975). The early CNS lesions caused by TMEV infection, characterized by degeneration of the motor neurones in the ventral horns of the spinal cord (Penney and

Wolinsky, 1979) have been used as a model for the study of the pathogenesis of lesions caused by poliovirus in man (Theiler, 1941; Olitsky, 1945; Lipton, 1975; Penney and Wolinsky, 1979; Rodriguez, Leibowitz, Powell and Lampert, 1983; Jubelt and Meagher, 1984). The later phases of the lesions caused by TMEV infection, with progressive demyelination (Lipton, 1975) have been intensively studied because of their similarities to chronic virus-induced demyelinating diseases of man, such as multiple sclerosis (Lipton, 1975; Lipton and Dal Canto, 1976a; Dal Canto and Lipton, 1977; Yamada, Zurbriggen and Fujinami, 1990).

The athymic (nu/nu) mouse has been widely utilized as a model for T lymphocyte deficiency. Experiments in these mice (Rosenthal *et al.*, 1986) indicated an important role for the immune system in limiting TMEV. When athymic mice are inoculated with TMEV, they develop severe hind limb paralysis at approximately 2 weeks post-infection, with most animals dying within the first month. The CNS lesions differ in several respects from those observed in immunocompetent mice. The neurones of the ventral horn, as well as those of the dorsal horn show spongiform degeneration. Because athymic mice lack humoral response to TMEV, oligodendrocytes undergo virus-induced vacuolar degeneration and lysis with rapid demyelination in the first week after infection (Rosenthal *et al.*, 1986).

Mice genetically homozygous for severe combined immunodeficiency (SCID) are severely deficient in both T and B lymphocytes, but have normal monocytes, macrophages and natural killer (NK) cell numbers and activity. SCID mice are highly susceptible to viral, bacterial and parasitic infections and, consequently, they are increasingly being used for research on the mechanism of resistance to infections (Bosma and Carroll, 1991). In the present paper, we report a spontaneous outbreak of TMEV infection in suckling SCID mice, as judged by the clinical signs, the serological findings and the histological lesions.

### Materials and Methods

The animal facility consisted of a number of self-contained barrier units. Each unit had several rooms. A breeding colony of SCID mice was kept in one room within one of these units, which also housed several strains of immunocompetent mice in other rooms. An in-house programme of health screening for viral pathogens was in operation. Testing for TMEV, however, was not included in this programme. The outbreak coincided with extensive building work carried out at the site in the proximity of the Animal House. Sick mice were killed by CO<sub>2</sub> inhalation immediately before post-mortem examination was carried out. All the blood samples were obtained by cardiac puncture immediately after death.

Necropsies were carried out on 14- to 18-day-old pups showing hind or front (one pup only) leg paralysis and on the mothers of these litters.

#### *Histopathology*

Multiple tissue samples from most organs, including brain and spinal cord, were immersion fixed in phosphate-buffered neutral 10 per cent formalin. After fixation, tissues were trimmed and routinely embedded in paraffin wax. Five- $\mu$ m-thick sections were cut and stained with haematoxylin and eosin (HE).

*Serology*

All sera sent to our laboratory during and after the outbreak were tested for the presence of antibodies against mouse hepatitis virus (MHV), minute virus of mice (MVM), lymphocytic choriomeningitis (LCM), reovirus-3, Kilham rat virus (KRV), H-1 Toolan and TMEV (GDVII Strain) by a modified ELISA method (Voller, Bidwell and Bartlett, 1976). In addition, serum samples from immunocompetent mice housed in the same and other units were obtained. Sample size was set to give a 95 per cent confidence level of detection of infection with a prevalence of 30 per cent or greater, following the recommendations of the National Research Council (Anonymous, 1976). Absorbance values were determined at 492 nm with an automatic 96-well spectrophotometer (Titertek). The results obtained for GDVII were confirmed by an immunofluorescence method for serum antibody detection which used BHK-21 cells infected with GDVII strain of TME virus (Smith, 1983).

## Results

*Clinical Signs and Gross Lesions*

Suckling mice of several litters became clinically sick and many died. Clinical signs included runting, depression, staring coat and unsteady walking. On average a third of the pups in each of the affected litters showed flaccid paralysis of the hind limbs or, less often, of the front limbs. All paralysed pups died within 2 to 3 days. Litter mates appeared sick and runted. Altogether, five litters in the room were affected. All the mice that showed clinical signs were less than 3 weeks old. Adults were not clinically affected.

Altogether eight paralysed or sick pups and three dams were necropsied. All pups had empty stomach and intestine, some had a very distended urinary bladder. No other gross lesions were found.

*Serology*

The results of all the serological tests carried out in the eight SCID mice were negative (1 in 10 dilution). The same serological tests were carried out on serum samples from immunocompetent mice contemporaneously housed in the same and the other units in the institution. Although the results varied from unit to unit, overall half of the sera tested were positive against GDVII only (1 in 10 dilution). The results of the corresponding immunofluorescent antibody tests were positive at 1 in 20 dilution. All the other tests remained negative in all samples tested throughout the duration of the outbreak.

*Histopathology*

The histological lesions found in the affected pups consisted primarily of degenerative changes in the spinal cord and brain stem.

The large motor neurones of the ventral horns of the spinal cord were the most severely affected. There was, however, a lesser involvement of the dorsal horns. The changes, which were seen at all levels of the cord, consisted of degeneration and necrosis of neurones and glial cells. Two types of degenera-

tion, as described by Penney and Wolinsky (1979), were observed. Infrequently (one to three per section), some shrunken neurones with dark cytoplasm and pyknotic nucleus (Fig. 1) were observed. Most degenerate neurones in the spinal cords of all the pups studied, however, displayed a striking spongiform appearance caused by extensive intracellular vacuolation. Some cells were distended to about four to 10 times their normal size (Figs 1, 2 and 3). Pyknotic nuclear remnants, including the nucleolus, were seen in the centre and some strands of pink material across this vacuolar space were all that remained of the cytoplasmic components (Fig. 2). Intracytoplasmic inclusion bodies were not found.

Many neurones showed milder forms of this vacuolation process, with several neatly circumscribed vacuoles within an otherwise normal cytoplasm or nucleus (Fig. 3). The nuclei of some neurones also appeared vacuolated (Fig. 2), although this was not as common as cytoplasmic vacuolation.

Vacuolar changes also affected glial cells, especially those in the ventral horns of the spinal cord. In some of these cells cytoplasmic vacuoles were small and crescent shaped. Other cells had the whole cytoplasm replaced by a large vacuole and a kidney shaped nucleus eccentrically compressed (Figs 1 and 3). Affected glial cells morphologically resembled astrocytes and oligodendrocytes.

In some cases, large vacuoles were found, surrounded by two or three compressed glial cell nuclei with dense-eosinophilic cytoplasm arranged around the vacuole, giving it a cystic appearance (Fig. 3). A few neurones were seen in the ventral horns of the spinal cord and in the brain stem, which showed chromatolytic degenerative changes (Fig. 4); some of these cells had a pale, ballooned cytoplasm, with eccentric nuclei, others did not have a visible nucleus. Altogether no more than 50 such cells were seen throughout all the sections studied.

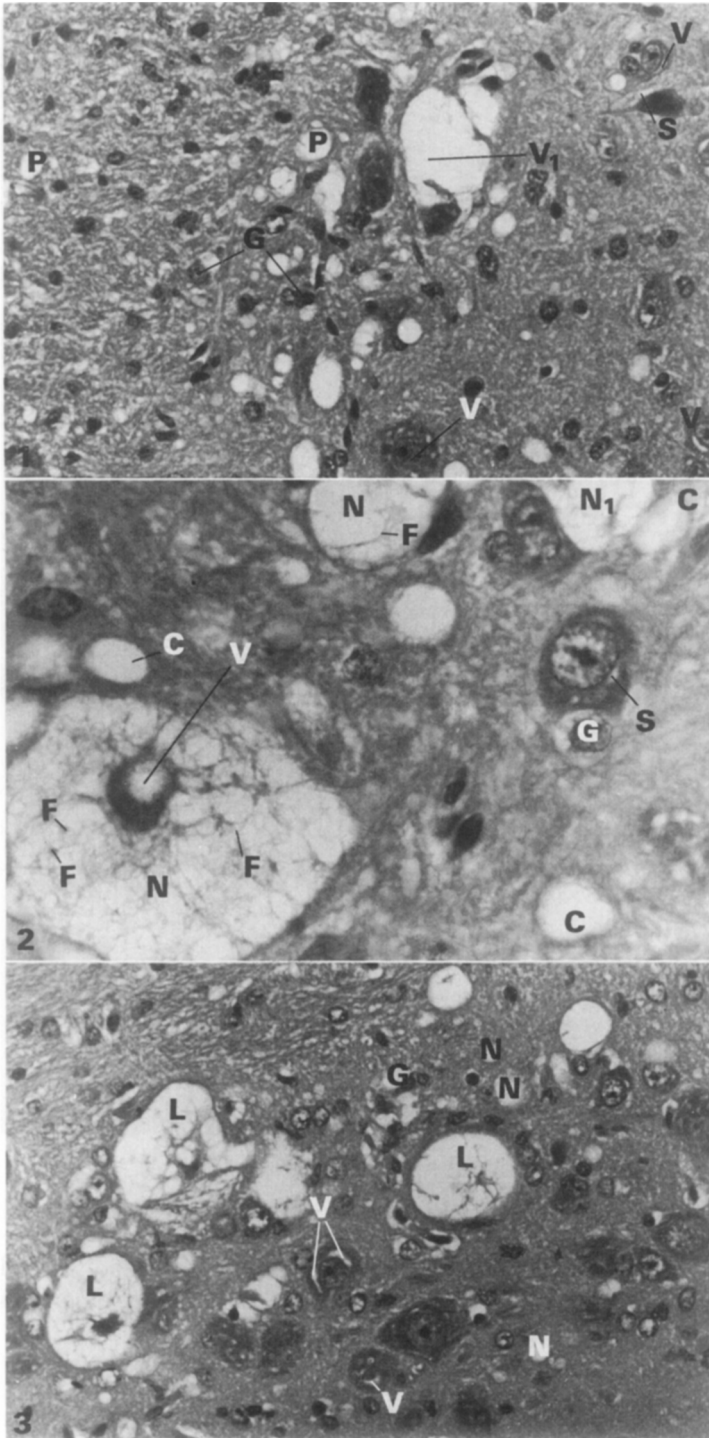
Lymphocytic inflammatory infiltrates, described as a major component of TMEV-induced changes in immunocompetent mice (Olitsky and Schlesinger, 1941), were conspicuously absent. Small groups of two to four glial cells around some vacuolated degenerating neurones (Fig. 3) were the only indication of a mild tissue response to the agent.

Discrete areas of the neuropil and of the white matter of the ventral column showed a microcystic appearance (Fig. 5). Similar changes were described by

Fig. 1. Cross section of the ventral horn of the spinal cord. V = Neurones at different stages of vacuolar degeneration; V1 = greatly distended neurone with dark, pyknotic nucleus; G = glial cells showing similar vacuolar changes; P = Extracellular vacuoles within the neuropil; S = shrunken degenerated neurone. HE  $\times$  310.

Fig. 2. High power section of the spinal cord. N = Vacuolar degeneration in neurones; F = thin cytoplasmic filaments; V = large vacuole in the nucleus which maintains a central position in the cell; N1 = another neurone at a milder stage of degeneration with a still well-preserved nucleus; G = similar changes in a glial cell; C = extracellular cystic spaces in the neuropil; S = a small cytoplasmic vacuole in another neurone. HE  $\times$  775.

Fig. 3. Section of the spinal cord. G = vacuole with compressed glial cell nucleus; V = small vacuoles in cytoplasm of neurones; N = vacuoles in the neuropil; L = enlarged neurones filled with vacuoles and remnants of nucleus and cytoplasm. HE  $\times$  310.



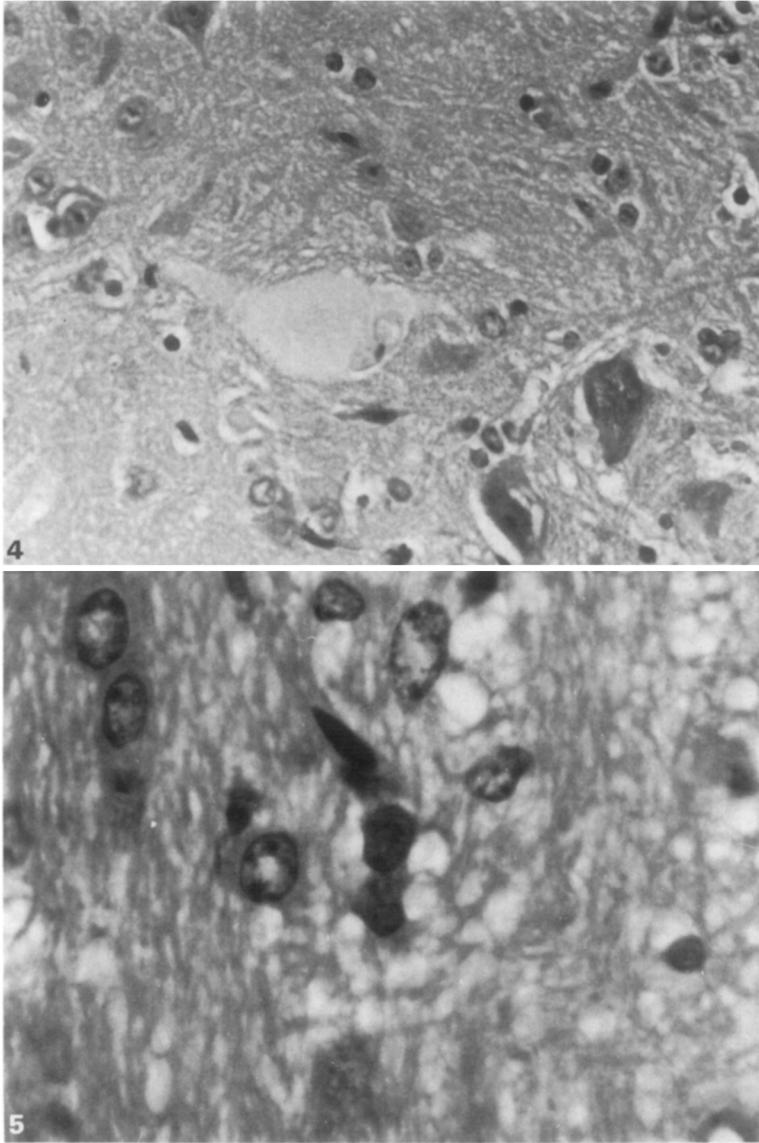


Fig. 4. Section of the spinal cord. Neurone showing chromatolysis. HE  $\times$  500.

Fig. 5. Microcystic changes in the white matter of the spinal cord. Longitudinal section. HE  $\times$  1250.

Lipton (1975) and attributed to vacuolation of the oligodendroglial loops surrounding axons (Rodriguez *et al.*, 1983). Luxol fast blue/Cresyl violet staining failed to demonstrate demyelination.

### Discussion

The basic lesions of encephalomyelitis involving the ventral horns and white matter in the spinal cord caused by TMEV are remarkably constant through-

out the numerous reports of experimental infections in mice (see references in the introduction). Degeneration and necrosis of neurones in the ventral horns of the spinal cord are considered pathognomonic of Theiler's encephalomyelitis (Lipton and Rozhon, 1986; Jacoby 1988).

In the present outbreak, the CNS localization of the microscopical lesions, together with the typical clinical signs and the serological evidence of TMEV infection in contemporaneous immunocompetent mice, provided clear evidence for a diagnosis of Theiler's encephalomyelitis.

Some of the lesions found in this outbreak, however, have not been described previously. The predominant histological feature was the presence of varying degrees of intracellular vacuolation; from small perinuclear vacuoles to large encysted spaces. This type of degeneration has only been described in a few of the reports on experimental TMEV infection (Penney and Wolinsky, 1979; Rodriguez *et al.*, 1983; Rosenthal *et al.*, 1986) all of which involved either newborn or nude mice. It is, however, a common feature of infection by many polio viruses in many different conditions (Burch and Harb, 1971; Burch, Tsui, Harb and Colcolough, 1971; Jubelt and Meagher, 1984). Furthermore, degenerative chromatolysis, as found in the neurones of the brain stem and spinal cord in this outbreak, has not been previously described in lesions caused by TMEV infection in mice. This feature is probably specific to the SCID mouse and as such is likely to be related to the immunological deficiencies of this strain.

The majority of mice found to have CNS lesions were under 3 weeks of age and all the paralysed animals died or were killed. Immediately following diagnosis the colony was culled. Consequently, we were only able to study lesions corresponding to the first (acute) phase of the disease during the lytic infection of CNS cells and not those which would develop if mice had survived to a later stage of chronic persistent infection with demyelination. Mild demyelinating changes have also been observed following TMEV infection of nude mice during the phase of lytic infection of neurones and of oligodendrocytes coinciding with scanty lymphocytic inflammatory infiltration (Rosenthal *et al.*, 1986). In the present outbreak there was evident vacuolar degeneration of glial cells and mild microcystic degenerative changes of the white matter, similar to those described by Lipton (1975). These changes occurred in the absence of lymphocytic cell infiltration.

Levine, Hardwick, Trapp, Crawford, Bollinger and Griffin (1991) have recently discovered that the persistent infection of the CNS of SCID mice with alphavirus can be cleared by treating the mice with specific antibodies from previously immunized congenic CB17 mice. Purified suspensions of T lymphocytes from similarly sensitized mice did not succeed in clearing the virus. A similar experimental approach applied to TMEV-infected SCID mice should assist in defining the contribution of the different lymphocytic subpopulations in the pathogenesis of demyelinating diseases of viral aetiology (Yamada *et al.*, 1990). SCID mice can be reconstituted by inoculation of mature lymphocytes, bone marrow or liver grafts from normal H2-identical BALB/c mice. These reconstituted models may prove of great value in understanding the role of the different immune mechanisms in the pathogenesis of disease.



Natural infection of mice with TMEV results in viral replication in the gastrointestinal mucosa. When high titre viraemia occurs, encephalitis ensues (Lipton and Rozhon, 1986; Jacoby, 1988). The mechanisms that determine these events are poorly understood (Zurbriggen and Fujinami, 1988; Brownstein *et al.*, 1989), but they are thought to resemble those that determine the occurrence of similar lesions during the course of poliovirus infection in man. The use of TMEV-infected immunocompetent or even nude mice as models of human poliomyelitis virus infection is not entirely satisfactory because in both cases intracerebral inoculation is required. Viral administration through this route bypasses all the defence mechanisms that render CNS infection an infrequent event in the natural disease.

Our finding that natural TMEV infections of SCID mice results in a very high incidence of clinical disease and severe degenerative lesions of grey and white matter indicates that this strain of mice could provide a useful model for the study of the mechanisms that determine invasion of the CNS and the modulation of the severity of the lesions following enterovirus infections. We also propose that immunological reconstitution of SCID mice infected with Theiler's encephalomyelitis virus might constitute an improved model for the investigation of mechanisms of virus-induced demyelination in man. Infection of highly sensitive suckling SCID mice, either by gavage or by natural transmission from experimentally infected dams, will offer a useful approach for the study of these mechanisms.

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