SUMMARY

Chronic measles encephalitis was induced in C57BL/6 mice with the hamster neurotropic strain of measles virus when virus which had been passaged at low dilution in the brains of suckling C57BL/6 mice was inoculated intracerebrally into 1- to 6-month-old mice. One-third to two-thirds of mice surviving the acute infection were consistently found to develop chronic neurologic dysfunction within 3 weeks to 1 year post-infection. The acute mortality was higher in males than in females and showed a slight decline with increasing age in males. Indirect immunofluorescence (IF) studies using measles virus-specific sera from a subacute sclerosing panencephalitis patient and from a hyperimmune rabbit demonstrated abundant viral antigen in regions of telencephalon and diencephalon correlated with the appearance of typical central nervous system signs in both acute and chronic disease. Viral antigen was found in infected neurons in the grey matter. Deposition of immune complexes was minimal as observed in adjacent brain sections stained with goat anti-mouse antibody by direct IF. Occasional necrotic foci and perivascular cuffing were observed in brains from chronically infected mice.

Although several animal models of chronic measles encephalitis have been described (for review, see Fraser & Martin, 1978), neither the mechanisms of measles virus persistence nor the immune responses to the virus have been completely characterized, partly because of the outbred nature of the hosts utilized. Chronic measles encephalitis in an inbred host was first described by Rammohan et al. (1980) with the hamster neurotropic (HNT) strain of measles virus in SJL/J mice. However, SJL/J mice are known to have altered immune functions (McFarlin & Waksman, 1982); therefore, in the present work, C57BL/6 mice were used to investigate the induction of chronic measles encephalitis by intracerebral (i.c.) infection with mouse-adapted HNT measles virus.

Suckling C57BL/6 mice (originally obtained from Olac, 1976 Ltd., Bicester, Oxon., U.K.) were used for HNT virus passages and adaptation as described in Burnstein et al. (1964). Briefly, 20% (v/v) suspensions of infected brains from moribund suckling mice were prepared in phosphate-buffered saline (PBS), pH 7.2, containing 2% (v/v) heat inactivated foetal calf serum, 150 U/ml penicillin and 150 μg/ml streptomycin. The adaptation of virus was monitored by increasing morbidity and mortality of suckling animals in successive passages. Virus stocks from the 3rd and 4th passages were used in the present work and were derived from a 2nd passage virus pool which produced maximum infectivity in suckling animals. The virus stocks were injected i.c. into 1- to 9-month-old C57BL/6 mice (0.03 ml/mouse). In the present study, the virus dose used was the amount needed to produce about 50% mortality (1 LD₅₀) in 2- to 3-month-old C57BL/6 mice. This was achieved by a 1:3 to 1:8 dilution of the stock viruses (passage 3 and 4) which contained 10² and 10²⁺ LD₅₀/ml respectively. In all, these virus stocks...
and doses were used for i.c. inoculation of approximately 1000 mice in about 50 experiments (range from six to 35 mice per experiment). Titration by plaque assay in BSC-1 and Vero cells showed that the passaged HNT virus stocks had low titres, $10^3$ and $3 \times 10^3$ p.f.u./ml respectively. HNT virus has about 100-fold lower virus titre in tissue culture systems when compared to titration in suckling animals (Burnstein et al., 1964). Mice used for control experiments were inoculated with uninfected suckling C57BL/6 mouse brain homogenate; there was neither death nor development of neurologic dysfunction during 12 months of observation.

Virus neutralization experiments were carried out in age- and sex-matched groups of adult mice (20 to 25 mice/group) inoculated i.c. with virus neutralized with 1/50 diluted, heat-inactivated subacute sclerosing panencephalitis (SSPE) serum after 3 h incubation at room temperature. The measles antibody titre of the SSPE serum was assayed at 1:4000 by the 50% plaque reduction test (Shirodaria et al., 1976).

Brains were removed intact from infected mice and halved; one half was stored at $-20^\circ$C after snap freezing in liquid nitrogen for immunofluorescence (IF) studies and the other fixed in Bouin's fluid for histology. Spinal cords and visceral organs were also collected from several sick mice for IF studies. Cryostat tissue sections, 5 μm thick, were cut, air-dried and fixed in acetone for 10 min for IF studies. For viral antigen detection, sections were incubated initially with either a rabbit anti-measles hyperimmune serum (indirect IF titre 1:400, diluted at 1:30) or an SSPE serum (indirect IF titre 1:8000, diluted at 1:50), and subsequently with commercial fluorescein isothiocyanate (FITC)-conjugated sheep anti-rabbit Ig or sheep anti-human IgG (Wellcome) respectively. All sera were absorbed with acetone-fixed HEp-2 cells, calf brain, mouse brain and liver to eliminate non-specific staining in control tissue sections. Staining for bound mouse Ig in immune complexes deposited in tissues was carried out by a direct IF procedure using a FITC-labelled goat anti-mouse Ig (Nordic Laboratories, The Netherlands) that had been absorbed with acetone-fixed HEp-2 cells and calf brain. Sections were incubated with immune serum for 40 min at 37°C in a humidified chamber, followed by two successive 10 min washes in PBS. Cryostat and paraffin-embedded brain sections were stained with haematoxylin–eosin (H&E) and Luxol fast blue (LFB) for histological examination. The determination of measles antibody titre in mouse sera was by the multilayer IF procedure as described by Chan (1984). Briefly, it was a three antibody layer indirect method, in which bound mouse globulin in the first layer was reacted with an unlabelled rabbit anti-mouse Ig (Nordic) at 1:10 dilution followed by a third layer of FITC-labelled sheep anti-rabbit Ig at 1:14 dilution.

The virus produced identical clinical effects in suckling mice or hamsters which showed inability to right themselves after being turned on their backs, hyperirritability, lethargy and progression to a moribund state with death in a day or less following the first signs of illness until all infected mice died within 4 to 6 days post-infection. However, i.c. inoculation of HNT measles virus into weanling or adult mice produced an acute clinical encephalopathy which began by the end of the first week, manifested by hyperactivity, seizures, convulsions, myoclonia, lethargy, inanition, coma and death. After acute mortality in which half of all mice inoculated with HNT died, delayed central nervous system (CNS) disease was consistently observed to develop in one-third to two-thirds of the remaining mice. The rest of the inoculated mice were asymptomatic throughout 16 months post-infection. In the present study, acute mortality was observed to be highest between 6 days and 12 days post-infection and mice with CNS signs after 3 weeks post-infection were considered chronically infected. The clinical manifestation of chronic infection varied from a protracted course, beginning at the acute phase of virus infection with continuous or intermittent display of CNS symptoms, to an insidious onset. Chronically infected mice displaying typical CNS signs were observed from as early as 3 weeks to over a year after inoculation. The general observation was sleepiness and reduced activity but paraplegia, lethargy, fear, extreme hyperirritability, seizures and altered behaviour were often associated.

About 5% of female mice infected at weanling or young adult age (1 to 3 months old) showed reduction in body length (measuring 65% of the length of control animals) after 6 or more months post-infection. This phenomenon has not been further explored in this present work. Perhaps it may be related to virus perturbation of pituitary functions as reported for lymphocytic
choriomeningitis virus (Oldstone et al., 1984), or, alternatively, to the production of autoantibodies against some endocrine hormones including growth hormone as observed in reovirus-infected mice (Haspel et al., 1983).

Clinical effects were absent in all mice inoculated with virus neutralized with SSPE serum suggesting that the effects in the absence of antiserum were specific for measles virus. However, suckling mice inoculated with a suspension of brain from chronically sick mice showed no morbidity and mortality and co-cultivation of mouse brains from acutely infected mice with Vero cells as described by Schumacher & Albrecht (1970) failed to demonstrate cytopathic effects characteristic of measles or the presence of specific measles IF in the tissue culture. Therefore, absence of infectious virus, or conversion to a defective form as reported previously by Roos et al. (1978) is suspected.

The acute mortality was slightly affected by both sex and increasing age of C57BL/6 mice as shown from results of 21 experiments (with an average of 17 mice per experiment) using the same virus infectious dose, namely 1 LD50 dose for 2- to 3-month-old C57BL/6 mice (Fig. 1). A moderate decline in acute mortality with age was observed in males (correlation coefficient significant at 1% probability level) but not in females. In several experiments using age-matched males and females, higher mortality in males than females was observed to be significant at 1:10 probability by $\chi^2$-test. The acute mortality observed for C57BL/6 mice in the present study differed from SJL/J mice (35% to 0%) as reported by Rammohan et al. (1980). The differences arising from age, sex and mouse strain may reflect different mechanisms of virus–host interaction.

Viral antigen localization in different regions of the brain was studied from serial cryostat sections stained by indirect IF, H&E and LFB. The viral antigen distribution was plotted on to a diagram of the brain section and the identification of brain regions was aided by the stereotaxic atlas of rat brain (Zeman & Innes, 1963). In infected mice, viral antigen was found to be abundant in cerebral cortex and basal ganglia. In addition, the hippocampus, thalamus, hypothalamus and occasionally the mesencephalon, pons, medulla and cerebellum were sparsely infected. The distribution of viral antigen in brains of mice with CNS disease sacrificed at various times post-infection is shown in Table 1. Studies of coronal sections of brains from two acutely sick mice at 8 and 9 days post-infection indicated bilateral distribution of viral antigen in the brain stem, basal ganglia and cerebral cortex, especially around the periventricular region.
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Table 1. *Measles virus antigen distribution in acutely and chronically infected mouse brains*

<table>
<thead>
<tr>
<th>Day post-infection of mouse sacrifice*</th>
<th>Clinical condition</th>
<th>Measles virus titre†</th>
<th>Virus distribution‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R  CC  H  BG  T  HY  MES  P&amp;M  CM</td>
<td></td>
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<tr>
<td>8</td>
<td>Seizures</td>
<td>4000</td>
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<tr>
<td>13</td>
<td>Hyperexcitability</td>
<td>2100</td>
<td>+ + + + + + - - ±</td>
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<td>16</td>
<td>Moribund, seizures</td>
<td>2100</td>
<td>+ + + + + + - - - -</td>
</tr>
<tr>
<td>23</td>
<td>Exanthema, lethargy, seizures</td>
<td>200</td>
<td>+ + + + + + ± + - -</td>
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<td>500</td>
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<td>60</td>
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<td>800</td>
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<td>86</td>
<td>Exanthema, lethargy, seizures</td>
<td>800</td>
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</tr>
<tr>
<td>182</td>
<td>Moribund, myoclonia, lethargy</td>
<td>1050</td>
<td>± + + + + + + ± ±</td>
</tr>
</tbody>
</table>

* Mice were injected when 1 to 3 months old with 1 LD₅₀ (for 2- to 3-month-old C57BL/6 mice: 0-03 ml i.c.) of the HNT strain of measles virus after 3 or 4 suckling C57BL/6 mouse brain passages.
† Determined by multilayer immunofluorescence procedure.
‡ Telencephalon = rhinencephalon (R), cerebral cortex (CC), hippocampus (H) and basal ganglia (BG); diencephalon = thalamus (T) and hypothalamus (HY); mesencephalon (MES); metencephalon = cerebellum (CM), pons and medulla (P&M); Scores = abundance of viral antigen (a), present (+), trace or doubtful (±) and absent (-).

In general, viral antigen appeared to concentrate in the grey matter whilst the white matter, choroid plexus, ependyma and meninges were spared. Infected neurons with the presence of viral antigen extending from the stroma of the cell body to the axons and dendrites could be identified morphologically (Fig. 2), but other infected cell types were not identified. In general, disseminated viral infection throughout the brain appeared to be more typical of chronically infected mice, whereas foci of infection in telencephalon and diencephalon were more common in acute and subacute illness. In the present work, the occurrence of viral antigen in brains has been established in all symptomatic mice examined (15/15 acutely sick mice and 15/15 chronically sick mice), but was absent in asymptomatic mice (0/5) and in one mock-infected mouse examined at 1 to 6 months post-infection. Viral antigen was not detected in cryostat sections of spinal cord, lung, liver and spleen of infected mice. Staining of deposited mouse immunoglobulin was minimal and diffuse in the region of viral antigen abundance in infected brain sections.

The correlation between histopathology and virus antigen distribution has not been thoroughly investigated. Preliminary studies with the LFB myelin stain have not shown up any prominent plaques of demyelination in chronic infected mouse brains but whether individual axons have demyelinated would require further study by electron microscopy. Several histological abnormalities, such as necrotic foci, perivascular cuffing and slight infiltration of inflammatory cells around the meninges, were sometimes present in the chronically infected brains studied.

Additional characteristics included the early induction of measles antibody in serum by the first week of infection and the increased incidence of anti-neurofilament autoantibodies during the delayed encephalitis which has been described by Chan (1984, 1985). According to Rammohan et al. (1981), the early induction of measles antibody may have a role in the establishment of chronic infection. Furthermore, the use of virus stocks passaged at low dilution may have facilitated the induction of chronic virus infection in this model, lending some credence to the possibility that interference by defective interfering particles in low-dilution inocula may account for the establishment and/or maintenance of measles virus persistence (Huang & Baltimore, 1970; Cernescu & Sorodoc, 1980).
An incidental observation in this HNT/C57BL/6 model pertaining to viral and immunopathological significance is the appearance of measles virus exanthem in some infected mice. This phenomenon will be described elsewhere (S. P. K. Chan, unpublished results). Briefly, the exanthem varied from mild maculo-papular rash and loss of hair to severe desquamation and the onset was between 3 weeks to a year post-infection. Furthermore, the condition is usually concurrent with typical CNS signs and measles-specific IF was demonstrated both in the brain and the skin lesions.

It is hoped that the model of chronic measles infection described in C57BL/6 mice will provide a valuable system for further research including studies of virus-associated autoimmunity, molecular mechanisms of measles persistence, and the design of possible therapeutic measures.

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REFERENCES

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