Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

ADVANCES IN VIRUS RESEARCH, VOL. 39

PATHOGENESIS OF THEILER'S MURINE ENCEPHALOMYELITIS VIRUS

Masahito Yamada, Andreas Zurbriggen, and Robert S. Fujinami*

Department of Pathology
University of California, San Diego
La Jolla, California 92093

I. Introduction and Historical Aspects

In 1933, Theiler found a young mouse with flaccid paralysis of the hind legs among the stock mice of his laboratory (Theiler, 1934, 1937). He investigated mice with the symptom and discovered that they were suffering from a new virus disease causing encephalomyelitis. This virus was similar to poliovirus in size, classified to the picornavirus family, and later referred to as Theiler murine encephalomyelitis virus (TMEV). Later, the original virus was called the TO (Theiler's original) strain. The virus could be recovered from feces and gastrointestinal tract of normal mice (Theiler and Gard, 1940b; Olitsky, 1940; Melnick and Riordan, 1947). This indicated that there were carriers of

* Present address: Neurology Department, Medical Center, University of Utah School of Medicine, Salt Lake City, Utah 84132

this virus in the breeding stock of "normal mice." Mice from noninfected, virus-free colonies were more susceptible to TO strain than the ordinary albino mice (Dean, 1951; von Magnus, 1951). Olitsky and Schlesinger (1941) pointed out similarities between TMEV and human poliomyelitis virus in clinical and pathological features, and considered the TMEV (TO strain) infection to be a mouse model for human poliovirus infection.

Besides the TO strain, other virus strains of TMEV with different characteristics have been isolated. Theiler and Gard (1940a) isolated GDVII and FA virus strains from mice during experiments with yellow fever virus. The two virus strains were highly virulent when compared with the TO strain, and caused severe meningoencephalitis and death (Olitsky, 1945).

Daniels *et al.* (1952) identified a new strain, called the DA (Daniels) strain which was similar to the TO strain. They found two kinds of lesions in older mice after intracerebral (ic) inoculation of the virus: acute polioencephalomyelitis and chronic demyelination of the spinal cord. This was the first description of demyelination induced by TMEV.

Later, Lipton (1975) focused on this characteristic biphasic disease process induced by the DA strain. The chronic demyelinating disease induced by the DA strain of TMEV shares several characteristics with the central nervous system (CNS) disease in multiple sclerosis (MS) and has been considered to be an animal model for MS. The features include (1) primary demyelination with inflammation in the CNS white matter, (2) pathological evidence of recurrent demyelination in certain mouse strains, (3) spastic paralysis caused by demyelination, (4) implication of immune-mediated demyelination, and (5) linkage to genetic background of the host (Lipton *et al.*, 1986). Major attention of the TMEV studies has been focused on the mechanisms of chronic demyelinating disease, including viral persistence and the role of the host immune response, and the molecular basis of neurovirulence of this virus. These problems will be discussed in this article.

II. Virology

A. Theiler's Murine Encephalomyelitis Virus Structure

Theiler's murine encephalomyelitis virus belongs to the family of picornaviridae. Picornaviruses are small ("pico"), phylogenetically related RNA viruses. Based on different biochemical and biophysical characteristics picornaviruses are subdivided into four groups: entero-

virus (including polioviruses, hepatitis A, and coxsackie virus), aphthovirus (foot-and-mouth disease virus), cardiovirus [encephalomyocarditis virus (EMCV), Mengo virus], and rhinovirus (human rhinovirus).

Theiler's murine encephalomyelitis virus was originally classified among the picornaviridae as an enterovirus because of its biological similarities with poliovirus (Rueckert, 1990). Theiler's murine encephalomyelitis virus is a naturally occurring enteric pathogen. However, the base composition of TMEV RNA determined by T1 ribonuclease digestion and gel electrophoresis was not typical for enteroviruses and lacked a poly(C) tract as found in cardioviruses (Rozhon *et al.*, 1982). More recent data obtained from sequence comparisons suggest that TMEV is more closely related to cardioviruses than to enteroviruses. Amino acid sequence studies of viral protein 1 (VP-1) of TMEV DA strain revealed a higher degree of similarities between TMEV and the cardiovirus EMCV than with other picornaviruses (Nitayaphan *et al.*, 1986). By sequencing 1925 nucleotides from the 5′ end of TMEV GDVII extensive similarities with EMCV were determined (Ozden *et al.*, 1986). Further comparison of the complete genome of TMEV BeAn 8386 strain identified remarkable similarities at the level of nucleotides and predicted amino acids between BeAn and the cardioviruses EMCV and Mengo virus (Pevear *et al.*, 1987a,b). However, anti-TMEV antibodies do not cross-neutralize with cardioviruses and may belong to a separate subgroup of cardioviruses.

Theiler's murine encephalomyelitis virus is a single-stranded nonenveloped RNA virus. The viral RNA is of positive sense, having the same polarity as mRNA. Viral mRNA lacks the cap structure found at the 5′ end of almost all eukaryotic mRNAs. The length of the picornavirus RNA varies from 7.2 to 8.5 kilobases (kb). The TMEV genome is about 8100 nucleotides long. All picornaviruses have noncoding regions at the 5′ and 3′ ends. The 3′ terminus is polyadenylated and the 5′ terminus is covalently linked by a phosphodiester bond to a virus-encoded protein VPg (Wimmer, 1979). The function of VPg is largely unknown. However, it has been suggested that VPg may function as a primer for RNA translation (Larsen *et al.*, 1980). The 5′ noncoding region differs in length from 610 nucleotides in human rhinovirus 1 (Skern *et al.*, 1985) to approximately 1200 in foot-and-mouth disease virus (Carrol *et al.*, 1984). The 5′ untranslated region of TMEV is 1065 to 1069 nucleotides long and lacks a poly(C) tract. A high degree of homology in the 5′ noncoding region within various groups of picornaviruses suggests a common ancestor. Viral positive-strand RNA can directly serve as template for virus transcription by a virally encoded RNA polymerase. The same polymerase is used to synthesize positive-

sense RNA copies which can then serve as mRNA for translation, as templates for further minus-strand synthesis, or be packaged into virions.

A large open reading frame between the two noncoding areas is translated into a polyprotein, which is then cleaved in a number of steps during and after translation to produce all the structural and nonstructural viral proteins. The TMEV open reading frame encodes a single polyprotein of about 2300 amino acids. This long polyprotein is subsequently cleaved into L, a small leader protein, present only in cardioviruses and aphthoviruses, P1, P2, and P3. The function of the 20 amino acid-long L protein is not yet identified. It may be important for virus replication. P1 is cleaved into the four structural proteins, VP-4, VP-2, VP-3, and VP-1. In addition much effort is concentrated on the function of the nonstructural proteins of picornaviruses. P2 is cleaved into three nonstructural proteins, 2A, 2B, and 2C, whose purpose for cardioviruses is not yet known. Recent observations suggest that the picornavirus proteinase 2A indirectly induces p220 cleavage, a component of the cap-binding complex, via modification of a proteinase of cellular origin (Lloyd *et al.*, 1988; O'Neill and Racaniello, 1989). This results in a loss of functional cap-binding protein activity. This event may prevent binding of cellular mRNA to ribosomes and consequently shut off the host cell protein synthesis after poliovirus infection. However, EMCV and TMEV do not encode this proteinase within 2A and do not cleave p220 *in vivo* (Lloyd *et al.*, 1988). In addition the TMEV strain, which was used for the above study, induces only a partial inhibition of host cell protein synthesis (Rodriguez *et al.*, 1987a). P3 is cleaved into four nonstructural proteins, 3A, 3B, 3C, and 3D, including a viral protease 3C, which is responsible for most of the posttranslational cleavage, and a viral polymerase 3D (Takahara *et al.*, 1989; Burns *et al.*, 1989; Roos *et al.*, 1989a). Theiler's murine encephalomyelitis virus protease 3C and polymerase 3D fusion proteins have been expressed in *Escherichia coli*. Antibodies to these proteins will allow further studies and comparisons of the function of nonstructural proteins among different TMEV strains (Ozden *et al.*, 1988).

Many recent picornavirus studies have been directed toward structure experiments and their relations to viral functions. All picornaviruses are icosahedral and contain 60 copies of each of the 4 structural proteins, VP-1, VP-2, VP-3, and VP-4, with molecular weights in the range of 37,000, 34,000, 27,000, and 6000 (Stroop and Baringer, 1981; Lipton and Friedmann, 1980; Lipton *et al.*, 1986). Recently, the three-dimensional structures of several picornaviruses have been resolved at the atomic resolution level: poliovirus as a member of the enteroviruses (Hogle *et al.*, 1985, 1987; Page *et al.*, 1988), foot-and-

mouth disease virus as a member of the aphthoviruses (Acharaya *et al.*, 1989), and Mengo virus as a member of the cardioviruses (Luo *et al.*, 1987). Results from the atomic structure of Mengo virus indicate that VP-1, VP-2, and VP-3 are external capsid proteins and VP-4 is completely internal (Luo *et al.*, 1987). VP-1 is the most external capsid protein on the surface of the virion. Analysis of the virus structure will further help to understand interactions between viral surface and antibody, and cell attachment. This information can be used in investigating molecular mechanisms of disease.

B. Differences among Theiler's Murine Encephalomyelitis Virus Strains

All TMEV strains are of the same serotype and cross-neutralize with polyclonal antisera (Lipton, 1978; Ohara and Roos, 1987). Based on different biological and pathological properties TMEV strains are divided into two subgroups: the most studied members of the TO subgroup (named after Theiler's original virus) are the DA, WW, and the BeAn 8386 strains. Members of the TO subgroup can cause a persistent demyelinating infection. The other subgroup consists of the highly virulent GDVII and FA strains, which cause an acute lethal polioencephalomyelitis. The differences between these subgroups are summarized in Table I and reviewed by Rodriguez *et al.* (1987a).

Recently the complete genomic sequence of members of both TMEV subgroups has been determined: GDVII as a member of the highly

TABLE I

DIFFERENCES BETWEEN TWO THEILER'S MURINE ENCEPHALOMYELITIS VIRUS SUBGROUPS TO (THEILER'S ORIGINAL) AND GDVII

Parameter	TO	GDVII
Members	DA, WW, BeAn, TO, Yale	GDVII, FA
Virulence	+	+++
Virus replication in CNS	+	+++
Acute disease	+/−[a]	++[b]
Chronic disease	+	−[b]
Virus persistence	+	−[b]
Plaque size	Small	Large
RNA synthesis *in vitro*	+	++
Cell tropism	Neurons, glial cells, macrophages	Neurons

[a]Depending on virus strain and host.

[b]Acute disease is normally lethal, therefore neither a chronic disease nor a persistence can be observed.

virulent subgroup (Pevear *et al.*, 1988a) and BeAn 8386 (Pevear *et al.*, 1987a) and DA (Ohara *et al.*, 1988) as members of the less virulent, demyelinating TO subgroup. The genome of GDVII consists of 8105 nucleotides with an open reading frame encoding 2303 amino acids. BeAn 8386 includes 8098 nucleotides and codes for 2303 amino acids, and DA has 8093 nucleotides and encodes 2301 amino acids.

Oligonucleotide fingerprinting of GDVII and DA RNAs showed significant differences (Rozhon *et al.*, 1982). However, tryptic peptide maps of the viral structural proteins indicated only minor differences between the highly virulent and the less virulent TMEV strains (Lorch *et al.*, 1984).

Nucleotide sequence comparisons of the highly virulent GDVII and the less virulent BeAn 8386 revealed a high degree of similarity: 90.4% of the nucleotides and 95.5% of the predicted amino acids were identical (Pevear *et al.*, 1988a). The closest identity was found within the two noncoding regions. In contrast, 39 of the amino acid variations were located within VP-1 (20), VP-2 (10), and VP-3 (9), the three external capsid proteins. Further characterization of the capsid protein differences between GDVII and BeAn 8386 was performed using a three-dimensional model (Pevear *et al.*, 1988b). Differences were observed in clusters within four specific regions on the surface of the virion. The putative virus receptor-binding site of both TMEV strains was highly conserved.

III. Pathology

A. In Vivo Infection

The pathologic features of the acute (early) and chronic (late) CNS diseases after TMEV infection are provided below.

1. Acute Disease

The acute disease found in the susceptible adult mice infected with DA strain is pathologically characterized by acute inflammatory lesions of the neurons (gray matter) in the brain and spinal cord (polioencephalomyelitis) with meningitis (Daniels *et al.*, 1952; Lipton, 1975). Anterior horn cells of the spinal cord and neurons of brain stem, hippocampus, and cerebral cortex are frequently involved (Fig. 1). Acute neuronal necrosis is associated with perivascular inflammation and microglial proliferation. Clinically, the acute disease following DA infection is characterized by flaccid paralysis of limbs due to lesions of the spinal anterior horn cells (Lipton and Dal Canto, 1976a).

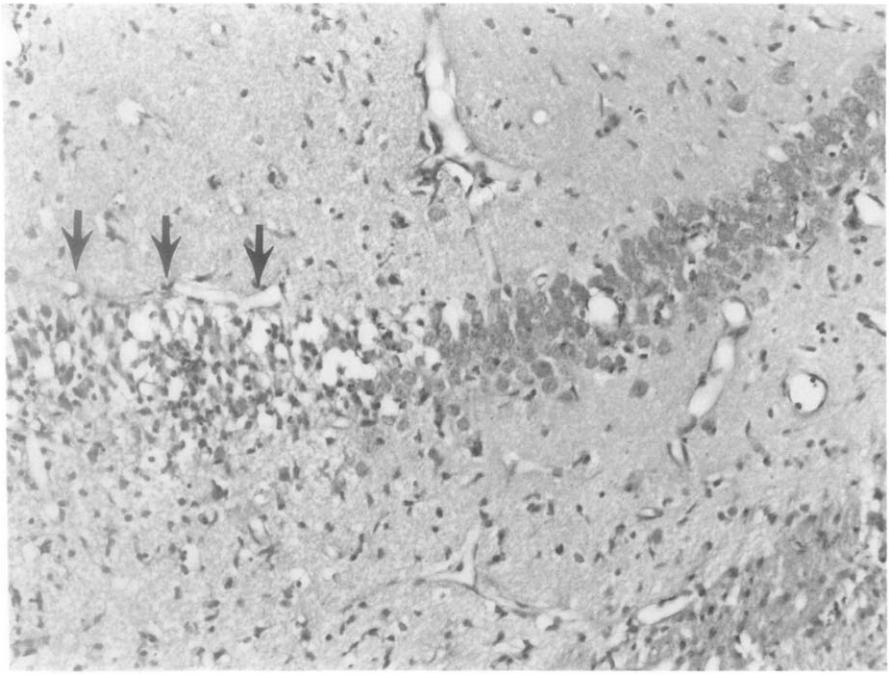

FIG. 1. Hippocampus from an SJL mouse 1 week after infection with DA strain of TMEV. Hippocampal pyramidal cells are showing acute necrosis (arrows). Hematoxylin–eosin stained. Magnification: ×150.

The mice, which could survive after the acute disease, are followed by the chronic disease.

GDVII and FA strains, the more neurovirulent strains, cause severe encephalitis, and most of the infected mice die soon after the onset of the symptoms (Theiler and Gard, 1940a; Olitsky, 1945; Stroop *et al.*, 1981).

When the DA strain or brain-derived WW strain were injected ic into neonatal mice, it also caused fatal encephalitis (Rodriguez *et al.*, 1983a; Wroblewska *et al.*, 1977; Penney and Wolinsky, 1979).

Electron microscopically, intracytoplasmic paracrystalline viral arrays were found in neurons of the CNS with acute encephalitis in WW- or DA-infected neonatal mice (Wroblewska *et al.*, 1977; Penney and Wolinsky, 1979; Rodriguez *et al.*, 1983a). In addition to neuronal infection, intracytoplasmic paracrystalline viral arrays were observed in oligodendrocytes (Penney and Wolinsky, 1979) and macrophages (Rodriguez *et al.*, 1983a) in acute lesions from WW- or DA-infected neonatal mice.

Immunohistochemically, viral antigens are found in neurons (gray matter) of the brain and spinal cord during the acute disease (Fig. 2). Viral antigens within neuronal processes in the acute gray matter lesions indicate the possibility of intraaxonal spread of the virus within the CNS (Rodriguez *et al.*, 1983a).

Viral tropism for neurons in the acute disease was demonstrated also by *in situ* hybridization analysis for viral RNA (Fig. 3) (Brahic *et al.*, 1981; Stroop *et al.*, 1981).

Thus, the acute CNS disease after TMEV infection is caused by direct viral infection of neurons.

2. Chronic Disease

In chronic phase, a month or more after infection with DA strain, inflammatory demyelinating disease occurs mainly in the white matter of the spinal cord and lingers for the life of the mouse (Daniels *et al.*, 1952; Lipton, 1975; Dal Canto and Lipton, 1975; Lehrich *et al.*, 1976). Extensive destruction of myelin sheaths with preservation of axons is found in the white matter of the spinal cord (Fig. 4). Mononuclear cell infiltration is observed in a perivascular or parenchymal distribution with inflammation of the spinal leptomeninges. De-

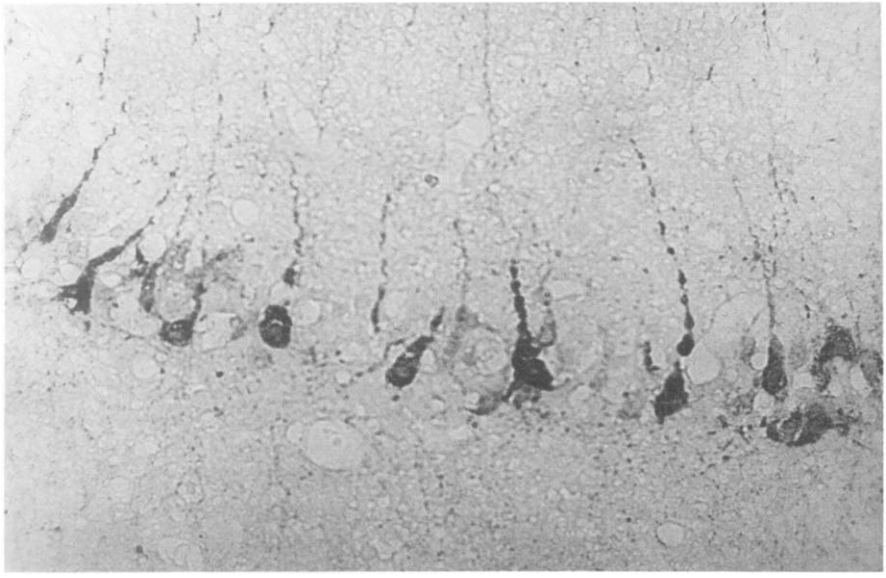

FIG. 2. Hippocampus from an SJL mouse 4 days after infection with GDVII strain of TMEV. The virus antigens are demonstrated in cell bodies and processes of the pyramidal neurons. Antibody to TMEV, ABC methods. Magnification: ×200.

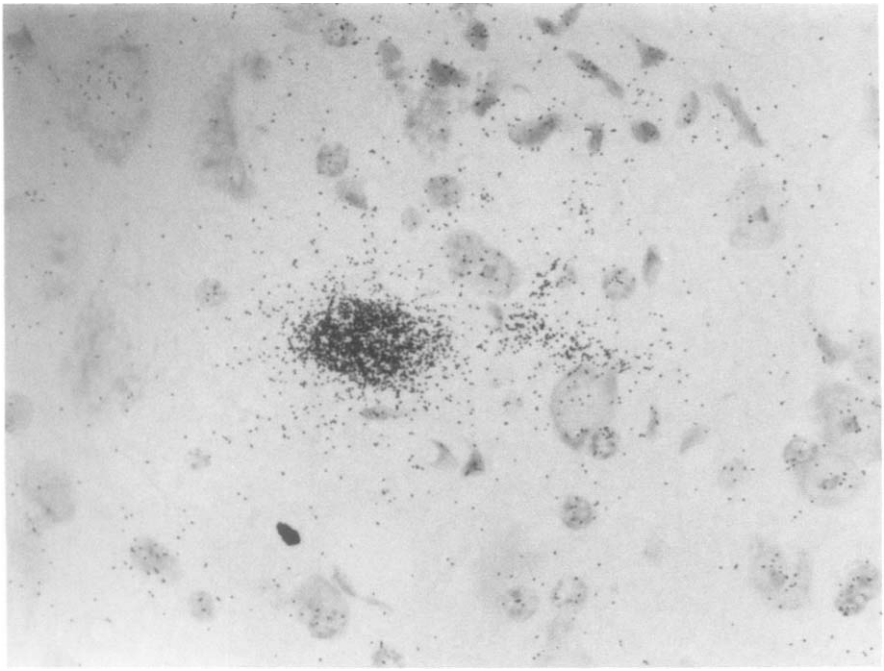

FIG. 3. Anterior horn of the spinal cord from an SJL mouse 1 week after infection with DA strain of TMEV. *In situ* hybridization with a cDNA probe for RNA of DA strain. Grains for the viral RNA are accumulated in a large neuronal cell, morphologically a motor neuron. Magnification: ×480.

myelinating lesions accompany many macrophages and astrocytosis. Clinical features of the chronic disease induced by DA strain infection include spastic paralysis of the limb, incontinence, and stimulation-induced prolonged extensor spasms of limbs caused by spinal cord white matter lesions (Daniels *et al.*, 1952; Lipton and Dal Canto, 1976b).

When tissue culture-derived WW strain is injected ic into outbred Swiss mice, a mild chronic inflammatory demyelination develops in the spinal cord without acute disease (Dal Canto and Lipton, 1980; Dal Canto, 1982; Dal Canto and Barbano, 1984). BeAn 8386 strain of TMEV also causes a chronic demyelinating disease without acute poliomyelitis in the susceptible mice (Lipton and Melvold, 1984).

Dal Canto and Lipton (1975) first described the ultrastructural pathology of TMEV-induced demyelination. Vesiculation of myelin and stripping of myelin lamellae by mononuclear cell processes were observed in the demyelinating lesions in the spinal cord from SJL mice

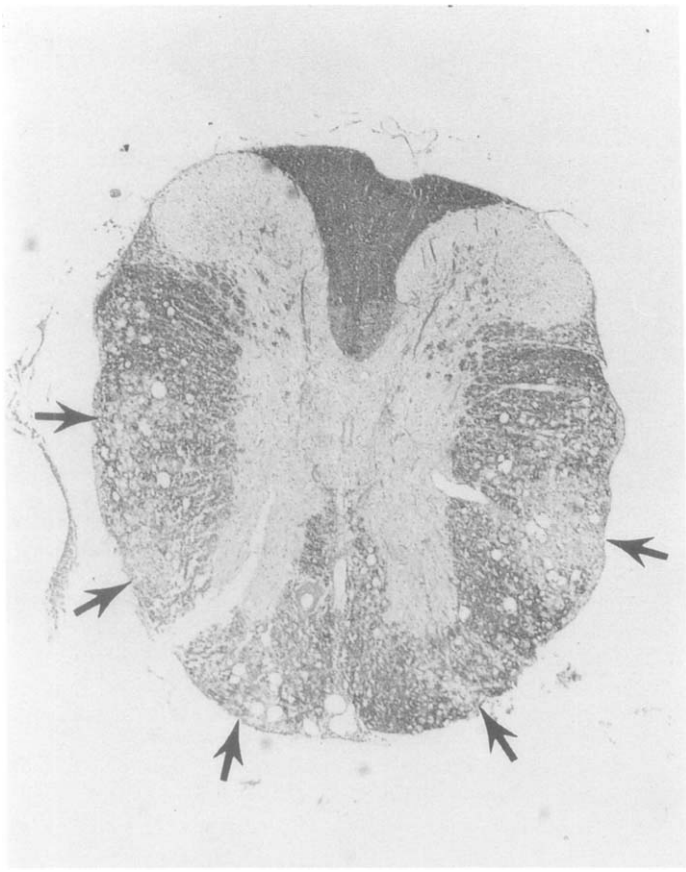

FIG. 4. Spinal cord from an SJL mouse 12 weeks after infection with DA strain of TMEV. Extensive demyelination (arrows) is found in the white matter. Antibody to myelin basic protein, ABC method. Magnification: ×50.

infected ic with DA strain. Demyelinating lesions were associated with inflammatory infiltrates consisting of lymphocytes, plasma cells, and macrophages. Oligodendrocytes in the lesions did not show degenerative changes or viral inclusions. They also performed ultrastructural immunohistochemical procedures to identify the cell infected with virus. In chronic demyelinating disease of the spinal cord, they observed viral antigens in macrophages, astrocytes, neuronal processes, but not in oligodendrocytes or Schwann cells which were remyelinating axons (Dal Canto, 1982; Dal Canto and Lipton, 1982). This suggested that demyelination was not caused by infection of myelinating cells.

In contrast, Rodriguez *et al.* (1983b) reported that most of the infected cells were oligodendrocytes in the chronic demyelinating disease from DA-infected SJL mice. Furthermore, Rodriguez (1985) reported that the earliest ultrastructural changes were in the inner cytoplasmic tongue of oligodendrocyte, the most distal part of the cell. He also observed the viral antigens in glial loops connecting with myelin lamellae. Blakemore *et al.* (1988) observed, in chronic demyelinating lesions of BeAn 8386-infected CBA mice, viral particles within oligodendrocytes as well as degeneration of oligodendrocytes and destruction and phagocytosis of myelin. These findings suggest that the viral infection of oligodendrocytes, myelin-producing cells, initiates myelin changes.

Remyelination was demonstrated electron microscopically in demyelinated areas of the spinal cord from WW-infected Swiss mice or DA-infected C3H/He mice (Dal Canto and Lipton, 1979, 1980). They found remyelination by Schwann cells, myelin-producing cells in the peripheral nervous system (Schwann cell-type remyelination), as well as by oligodendrocytes. Schwann cell-type remyelination was dominant in the outer white matter of the spinal cord and seemed to be related to destruction of the glial limiting membrane caused by the infiltration of inflammatory cells (Dal Canto and Barbano, 1984).

In situ hybridization studies using a cDNA probe for viral RNA have demonstrated viral presence in the spinal cord white matter during the chronic demyelinating disease (Brahic *et al.*, 1981; Stroop *et al.*, 1982). Further, they found that the degree of inflammation in the spinal cord white matter paralleled the number of cells containing the viral RNA (Chamorro *et al.*, 1986). They also reported that, of the viral RNA-containing cells, 25–43% were oligodendrocytes, 5–10% were astrocytes, 10% were microglia/macrophages, and the rest were unknown, as identified by double-labeling methods using *in situ* hybridization for the viral RNA and immunohistochemistry (Aubert *et al.*, 1987). These results indicated that oligodendrocytes can be a major target for TMEV infection, and that the virus infection of oligodendrocytes may be related to pathogenesis of the chronic white matter disease.

We investigated the spatial and chronological relationships between viral RNA, myelin-specific mRNAs, and demyelination in mice infected with DA strain by using *in situ* hybridization and slot-blot analyses (Yamada *et al.*, 1990b). In the spinal cord, 1 to 2 weeks postinfection (pi), viral RNA was commonly detected in ventral root entry zones of the white matter as well as in anterior horn neurons (Fig. 3). Viral RNA within the spinal cord white matter increased to maximum levels by 4 weeks pi with inflammation and mild demyelination (Fig. 5). Later, by 8 to 12 weeks pi when demyelination was very extensive,

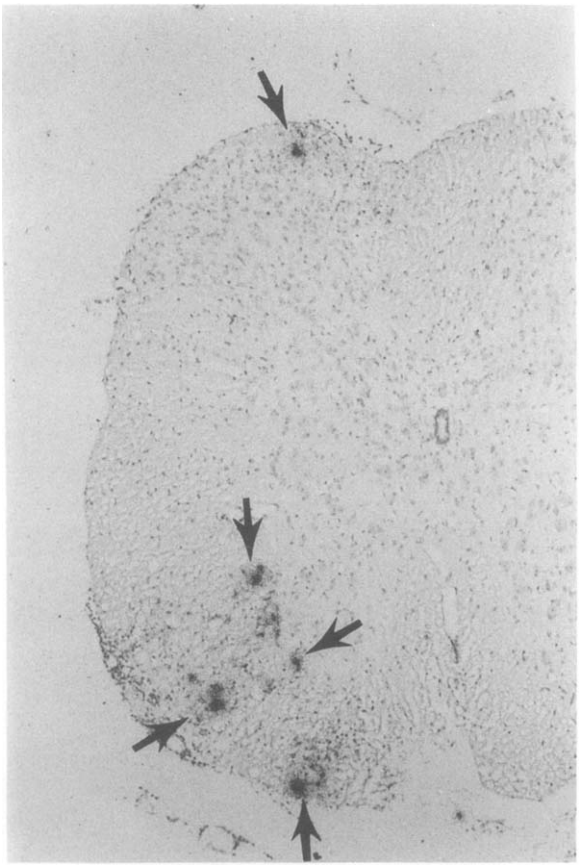

FIG. 5. Spinal cord from an SJL mouse 4 weeks after infection with DA strain of TMEV. *In situ* hybridization with a cDNA probe for RNA of DA strain. Viral RNA is distributed in the white matter (arrows). Magnification: ×75.

viral RNA significantly decreased. Demyelination was associated with inflammation, but did not parallel the presence of viral RNA within the spinal cord. A decrease of myelin-specific mRNAs, including myelin basic protein (MBP) and proteolipid protein (PLP) mRNAs, was observed within the demyelinating lesions with or without detectable viral RNA, indicating suppression of the oligodendroglial activity for maintenance and/or reproduction of myelin. Our results indicate that a viral infection of white matter in the early phase of the infection initiates spinal cord disease, leading to demyelination. However, dur-

ing the chronic phase (8–12 weeks pi) of the disease, an indirect immunopathological process could contribute to the presence of extensive demyelination.

Thus, virus can infect some glial or inflammatory cells of the white matter and lead to demyelination, although there is still controversy concerning what kinds of cells are infected with virus in the lesions. It is unlikely that the demyelination during the chronic phase of infection is simply attributed to direct viral infection of myelin-producing cells, oligodendrocytes.

B. In Vitro Infection

Since TMEV was first propagated in mouse embryo brain cell cultures by Parker and Hollender (1945), TMEV has been cultured with CNS and non-CNS cells. Currently, TMEV is commonly propagated in BHK-21 (baby hamster kidney) cell and L2 cell cultures. Cellular pathology after infection with TMEV *in vitro* has been described (Powell *et al.*, 1977; Wroblewska *et al.*, 1979; Graves *et al.*, 1986; Frankel *et al.*, 1986).

In BHK-21 cells infected with DA strain, Powell *et al.* (1977) observed viral cytopathic effects and intracytoplasmic crystals composed of 25-nm virus particles.

Wroblewska *et al.* (1979) infected myelinated CNS organotypic cultures from explants of newborn mouse CNS with WW strain. They observed neuronal degeneration, demyelination, and cytoplasmic inclusions containing viral paracrystalline arrays in astrocytes. Graves *et al.* (1986) found, in primary brain cell cultures derived from newborn mice or embryos and infected with DA strain, a lytic infection of neurons and oligodendrocytes, and persistent infection of astrocytes and macrophages.

Frankel *et al.* (1986) infected Schwann cell cultures from mouse sciatic nerves with GDVII and WW strains. Schwann cells produce myelin in the peripheral nervous system. They found a cytopathic effect (rounding up of cells) of Schwann cells and viral antigens in their cytoplasm. By electron microscopy, GDVII virions were found in all infected cells. In contrast, WW virions were found in only a few cells. This finding indicated differences in the development of both viruses in Schwann cells.

These data suggest that infection and degeneration of myelin-producing cells (oligodendrocytes and Schwann cells) and demyelination can occur in *in vitro* conditions in the absence of functional immune system.

IV. Immunology and Immunopathogenesis of Theiler's Murine Encephalomyelitis Virus-Induced Demyelination

Immune responses during TMEV infection have been considered to have two opposite roles in TMEV-induced CNS disease: One is a protective role to limit virus replication, eliminate virus, and suppress the acute encephalitis due to the viral cytopathic effects. Spinal cord white matter disease is often associated with persistent viral infection as discussed in Section III. Therefore, escape of the virus from the immune system and its persistence within the spinal cord is a critical issue for demyelination during the chronic phase. The other is an immune-mediated basis for demyelination. The immune-mediated hypothesis for TMEV-induced demyelination was originally proposed by Lipton and Dal Canto (1976b, 1977). Treatment of TMEV-infected mice with immunosuppressive agents resulted in a decrease of demyelination (Lipton and Dal Canto, 1976b, 1977).

In this section, we discuss immunologic aspects of the virus-induced disease, focusing mainly on the immunopathogenesis of demyelination.

A. Immunogenetics

The susceptibility to demyelination in TMEV infection is variable, depending on the mouse strain (Lipton and Dal Canto, 1979). For example, when mice were injected with the DA strain, demyelination was found to be severe in SJL/J mice, and minimal or absent in C57BL/6 or BALB/c mice (Lipton and Dal Canto, 1979). Later, immunogenetic analyses have revealed that susceptibility to demyelination is related to multiple genes, including the major histocompatibility complex (MHC) genes and non-MHC genes (Lipton and Melvold, 1984; Clatch *et al.*, 1985, 1987a; Rodriguez and David, 1985; Rodriguez *et al.*, 1986a; Melvold *et al.*, 1987).

At least one of the genes related to disease susceptibility is linked to the H-2 complex, and the D region (H-2D) strongly influences demyelination (Lipton and Melvold, 1984; Clatch *et al.*, 1985, 1987a; Rodriguez and David, 1985; Rodriguez *et al.*, 1986a; Melvold *et al.*, 1987). This fact indicates that class I-restricted [$CD8^+$ (cytotoxic/suppressor)] T cells may be critical for susceptibility to demyelination due to the association with H-2D, a class I region determinant. Class I antigens are expressed in the spinal cord of TMEV-infected mice (Lindsley *et al.*, 1988). Class I gene products can act as restricting elements for $CD8^+$ T cells. Variations in class I-restricted cytotoxic T cell responses or alternations of class I-restricted suppressor T cell

function may be important for the pathogenesis of demyelination (Rodriguez *et al.*, 1986a; Clatch *et al.*, 1987a). Dysfunction of a cytotoxic T cell immune response to infected brain cells may lead to persistence of virus infection. Impairment of suppressor T cell function may allow damage to myelin or oligodendrocytes mediated by class II-restricted [$CD4^+$ (helper or delayed hypersensitivity type)] T cells. Another gene related to susceptibility for TMEV-induced demyelinating disease is closely linked to a gene encoding the β chain of the T cell receptor (Melvold *et al.*, 1987). Expression of class II antigens was found in glial and endothelial cells of the susceptible mice infected with DA virus, but much less in DA virus-infected resistant mice, suggesting the importance of class II gene products and class II-restricted T cell responses responsible for demyelination (Rodriguez *et al.*, 1987b).

Thus, susceptibility to the disease development (demyelination) is closely associated to genes regulating the immune system. Further studies are necessary to clarify loci of genes involved and their role in pathogenesis.

B. Effects of Immunosuppression on Demyelination

Lipton and Dal Canto (1976b, 1977) first demonstrated that immunosuppression by cyclophosphamide or anti-thymocyte serum decreased demyelination induced by TMEV, suggesting an immune-mediated mechanism of demyelination. In those mice, both the humoral and cellular immune responses to the virus were suppressed. Later Roos *et al.* (1982) demonstrated that only a short-term immunosuppressive regime with these agents at the time of virus injection was sufficient to decrease demyelination.

Treatment with antibodies to MHC-encoded Ia (class II) molecules has been reported to decrease demyelination in SJL/J mice infected with the DA or WW strain (Rodriguez *et al.*, 1986b; Friedmann *et al.*, 1987). Depletion of $L3T4^+$ ($CD4^+$) T cells [helper/delayed-type hypersensitivity (DTH) T cells] by treatment with antibodies to L3T4 prior to the onset of clinical signs resulted in a marked decrease of the demyelination in CBA mice infected with BeAn (Welsh *et al.*, 1987). These data suggest a role for $CD4^+$ (helper/DTH), class II-restricted T cells. In contrast, Rodriguez and Sriram (1988) reported that depletion of $Lyt\text{-}2^+$ ($CD8^+$) T cells (cytotoxic/suppressor T cell) with anti-Lyt-2 antibody resulted in a decrease of demyelination. MHC H-2D region is related to susceptibility to the disease as discussed above. These results suggest that demyelination is mediated by $CD8^+$ (cytotoxic/ suppressor), class I-restricted T cells.

Thus, immunosuppression experiments have disclosed that TMEV-induced demyelination is mediated by immune system. Critical roles for $CD4^+$, class-II restricted T cells and/or $CD8^+$, class-I restricted T cells are postulated.

C. Cellular Immunity and Demyelination

Lipton, Miller, and colleagues have reported that demyelinating disease in TMEV infection is highly correlated with class II-restricted, TMEV-specific delayed-type hypersensitivity (DTH) responses (Rabinowitz and Lipton, 1976; Clatch *et al.*, 1986, 1987b). They have proposed that $CD4^+$ ($L3T4^+$, $Lyt\text{-}1^+2^-$) class II-restricted T_{DTH} cells activate macrophages through lymphokine release, and the activated macrophages cause a nonspecific destruction of myelin tissues (innocent bystander theory).

Lindsley and Rodriguez (1989) characterized T cell responses in the CNS of mice with TMEV infection. They demonstrated an increase of $Lyt\text{-}2^+$ ($CD8^+$) cells during demyelination, and parenchymal distribution of $Lyt\text{-}2^+$ ($CD8^+$) cells, but not of $L3T4^+$ ($CD4^+$) cells, in the demyelinating lesions. This suggests that antiviral or autoimmune $CD8^+$ T cells play a critical role for demyelination if the target antigens are presented in the context of class I molecules on myelin or myelin-producing cells, oligodendrocytes.

Natural killer cell activities have been reported to be critical in protecting infected mice against TMEV-induced gray matter disease, but not linked to demyelinating disease of the white matter (Paya *et al.*, 1989).

From the similarities with the demyelinating pathology found in experimental allergic encephalomyelitis (EAE), cellular immune responses directed at myelin or other CNS components and adoptive transfer experiments of spleen cells have been explored. Barbano and Dal Canto (1984) reported that splenocytes from TMEV-infected mice showed no proliferative responses to mouse spinal cord homogenate or MBP and that passive transfer of these cells to naive mice failed to produce disease. Miller *et al.* (1987) found no T cell responses directed at mouse spinal cord homogenate or major myelin proteins, including MBP and PLP. Treatment of SJL/J mice with myelin components prior to TMEV infection did not affect the development of the demyelinating disease, in contrast to EAE, where such treatments could prevent the disease (Lang *et al.*, 1985). These results suggest that the immune-mediated process of TMEV-induced demyelination is different from that of EAE.

In contrast, Rauch and Montgomery (1988) reported that adoptive

transfer of spleen cells from infected mice could facilitate a demyelinating disease in naive recipients, although transfer of the virus could not be ruled out.

Combined with the results of the immunogenetic analysis and immunosuppression experiments, the data of cellular immunity indicate that T cell immune responses to the virus in association with MHC antigens have important roles in demyelination, although the exact effector mechanisms for demyelination remain to be determined. A possible role of T cell immune responses to self-antigen(s) of CNS in demyelination also requires further investigation.

D. Humoral Immune Response and Demyelination

Humoral immune responses to TMEV play a critical role in the elimination of TMEV from the infected mice. Antibodies to the virus are found in sera and cerebrospinal fluid (CSF) of infected mice, and intrathecal production of the antibodies was reported (Lipton and Gonzalez-Scarano, 1978; Roos *et al.*, 1987; Rodriguez *et al.*, 1988). Cerebrospinal fluid oligoclonal IgG bands, which are known to appear in patients with MS, were detected in mice infected with TMEV (Roos *et al.*, 1987; Rodriguez *et al.*, 1988). IgG-containing cells were found in the inflammatory cells of the CNS (Lipton and Gonzalez-Scarano 1978; Rodriguez *et al.*, 1988). IgG secreted by B cells which were isolated from the infected CNS reacted with VP-1 and VP-2 of the virus, and had neutralizing activity. Theiler's murine encephalomyelitis virus neutralizing antigenic sites are mainly located on VP-1 of the virus (Nitayaphan *et al.*, 1985a,b; Clatch *et al.*, 1987c) (see Section II). Treatment with neutralizing antibody to VP-1 reduced the virus and improved survival of mice (Fujinami *et al.*, 1989).

The presence and pathologic roles of autoreactive antibodies to myelin components have been investigated to test a possibility of autoimmune demyelination by such antibodies. Barbano and Dal Canto (1984) described that serum from TMEV-infected mice did not injure myelinating cultures. Rodriguez *et al.* (1988) reported that IgG in the serum and CSF of the infected mice did not bind to myelin or other CNS components and that IgG or complement were rarely found on myelin sheaths. These data suggest the absence of autoantibodies to CNS components in TMEV infection. In contrast, Raunch *et al.* (1987) reported the appearance of MBP in CSF and serum antibody directed against MBP. Anti-MBP antibody has been suspected to be secondarily generated by immune responses to degraded myelin (Raunch *et al.*, 1987) because there has been no evidence of cross-reaction between TMEV proteins and MBP (Rauch *et al.*, 1987; Rubio and Cuesta, 1989).

Cash *et al.* (1989) reported that IgG secreted from B cells isolated from the CNS of infected mice reacted with nonviral white matter components as well as viral proteins. Such components were present only in infected animals.

We have discovered that one of the monoclonal antibodies (MAbs) raised against TMEV (DA strain), H8, reacts with galactocerebroside (GC), a major lipid component of myelin, as well as VP-1 (Fujinami *et al.*, 1988). MAb H8 reacted with oligodendrocytes in brain cell cultures and myelin structures in frozen brain sections (Yamada *et al.*, 1990a). When injected into mice with EAE, MAb H8 increased the demyelination by 10-fold (Yamada *et al.*, 1990a). The sera from mice with chronic TMEV infection had antibodies to GC as well as to TMEV as determined by enzyme-linked immunoabsorbent assay (ELISA). Furthermore, a competition ELISA for TMEV or GC antigen revealed that the sera from these infected mice contained antibody(s) with the same or similar specificity as MAb H8 (Yamada *et al.*, 1990a). These data suggest that immune responses to TMEV can generate antibodies to GC, and contribute to TMEV-induced demyelination through immune-mediated processes.

Humoral immune responses to TMEV generate neutralizing antibodies within the CNS which can aid in virus clearance. In relation to demyelinating disease during the chronic phase, autoantibodies directed at myelin components can be generated, and contribute to demyelination. Development of autoantibodies may be based on molecular mimicry between virus and self component, as suggested in our studies (i.e., common determinant between VP-1 of the virus and GC of myelin) (Fujinami *et al.*, 1988; Yamada *et al.*, 1990a). Other possible mechanisms for virus-induced autoimmunity include viral infection of immune cells resulting in aberrant responses to virus and self determinants, polyclonal B cell activation, and generation of antiidiotype responses (Fujinami and Zurbriggen, 1989), as well as altered self.

E. Theiler's Murine Encephalomyelitis Virus Infection of Nude Mice

To elucidate the role of the immune system in the development of demyelination induced by TMEV, the athymic nude mouse, a model for T lymphocyte immunodeficiency, has been used for TMEV infection. These animals fail to produce anti-TMEV antibodies as well as sensitized T lymphocytes (Roos and Wollmann, 1984; Rosenthal *et al.*, 1986). These animals provide a good tool to analyze a direct viral effect in the absence of a functional immune system.

When BALB/c nude mice were infected ic with the DA strain, demyelinated foci were observed in the spinal cord (Roos and Wollmann,

1984; Rosenthal *et al.*, 1986). In addition, infections of oligodendrocytes as well as of neurons were reported as demonstrated ultrastructurally or immunohistochemically (Rosenthal *et al.*, 1986). These data suggest that the viral lytic infection of oligodendrocytes is sufficient to produce demyelination in immunodeficient mice.

Furthermore, the route of the virus inoculation did not affect the distribution of the lesions in DA-infected nude mice (Love, 1987). Zurbriggen and Fujinami (1988) demonstrated the presence of viral RNA in vascular endothelial cells as well as in neurons and glial cells by using *in situ* hybridization in DA virus-infected nude mice. In these animals, the virus in the blood may enter the CNS by infecting vascular cells and crossing the blood–brain barrier. In turn, virus growing in the CNS can reseed the blood stream. This also suggests that the vascular infection of endothelial cells may be important for antigen presentation of viral peptides to the immune system in immunocompetent mice (Zurbriggen and Fujinami, 1988).

V. Theiler's Murine Encephalomyelitis Virus Variants to Study Pathogenicity

The nucleotide sequence of members of both TMEV subgroups has been identified. Nucleotide sequence comparisons of the highly virulent versus the less virulent TMEV strains have revealed a high degree of similarity. However, although only few amino acids differed, there are still too many nucleotide sequence differences between the virulent and the less virulent TMEV strains to identify regions of the viral genome responsible for differences in pathogenicity of TMEV. Therefore we studied in our laboratory virus variants with an altered pathogenicity, derived from the same virus strain (Zurbriggen and Fujinami, 1989). Recent observations have shown that antigenic mutants from various viruses can be selected using neutralizing MAbs. Neutralizing MAbs have been used to select less neurovirulent reovirus variants (Spriggs and Fields, 1982). Similarly, neutralization-resistant coronavirus variants with an altered pathogenicity have been obtained (Fleming *et al.*, 1986; Dalziel *et al.*, 1986). The use of neutralization-resistant TMEV variants has been confirmed by Roos *et al.* (1989c).

VP-1, the most external viral capsid protein, is highly accessible to antibodies and plays an important role in TMEV infection. VP-1 also contains a major neutralizing epitope (Nithayaphan *et al.*, 1985a,b). Therefore, to define the role of TMEV VP-1 in modulating CNS disease, we selected neutralization-resistant TMEV variants using a

MAb H7 (Zurbriggen and Fujinami, 1989). On Western immunoblots with purified TMEV proteins MAb H7 reacted with VP-1 and in neutralization assays MAb H7 neutralized TMEV DA strain. A virus variant, H7A6-2, which escaped neutralization by MAb H7, was inoculated ic into susceptible SJL/J mice and the resulting pathology and virus replication was compared to the parental DA strain over a period of 12 weeks. An altered and diminished disease pattern within the spinal cord versus the parental DA strain was observed.

Variant virus H7A6-2 produced significantly less inflammatory lesions in the CNS. The inflammatory response in the spinal cord of variant virus-infected mice peaked by 2 weeks postinfection (pi), whereas the inflammatory lesions in the parental DA virus-infected mice gradually increased in size and number until approximately 8 weeks pi.

In addition, a considerable smaller number of infected cells in the spinal cord were detected in mice infected with the virus variant H7A6-2. The number of infected cells in the variant virus-infected spinal cords peaked at 1 week pi and then gradually declined. Already by 2 weeks pi a few infected cells were demonstrable in the spinal cord of variant virus-infected mice. However, the number of DA wild-type virus-infected cells steadily increased until 8 weeks pi.

The pattern of viral antigen distribution, as shown by immunohistochemistry using rabbit hyperimmune sera to TMEV, was similar between wild-type and variant virus. Equal results were obtained using *in situ* hybridization (Zurbriggen and Fujinami, 1988).

The amount of infectious virus in the spinal cord of wild type- versus variant virus-infected mice was determined by plaquing spinal cord tissue homogenates on BHK-21 cells. Similar amounts of infectious virus could be detected at 3 days pi in DA wild type- and variant virus-infected mice. Infectious variant virus steadily declined thereafter and at 4 weeks pi no infectious virus was demonstrable. In contrast, spinal cord tissue from mice infected with the parental DA virus contained infectious particles throughout the observation period. In addition, isolated variant virus retained the H7 phenotype and was resistant to neutralization by MAb H7 (Zurbriggen and Fujinami, 1988).

Variant virus H7A6-2 was further characterized by ELISA, neutralization assay, and by sequencing selected regions of its RNA genome (Zurbriggen *et al.*, 1989). ELISAs were performed using two neutralizing MAbs, H8 and H7, and a nonneutralizing MAb, H5. All these MAbs recognize VP-1 on Western immunoblots. As antigens for the ELISA we used three different virus strains, GDVII, the parental DA wild type, and the H7A6-2 virus variant (Zurbriggen *et al.*, 1989). MAbs H5 and H8 recognized all three tested viral antigens. However,

MAb H7, which was used to select the virus variant, bound only to the parental DA virus.

The results from the ELISA were supported by neutralization assays. Monoclonal antibody H8 neutralized all three virus strains, whereas MAb H7 neutralized only the parental DA virus. These results indicated that there are at least two different neutralizing epitopes within VP-1 and that the H7A6-2 virus variant has been changed at an epitope specific for the DA strain.

In addition no major changes in the migrational characteristics of H7A6-2 VP-1 could be observed. No differences in the migration of the structural viral proteins were observed on SDS-PAGE between DA and H7A6-2 virus. Thus, there were no major deletions in the variant virus proteins (Zurbriggen *et al.*, 1989).

Direct RNA sequencing revealed only one single nucleotide difference in the region encoding VP-1 between the variant virus and the parental DA virus (Zurbriggen *et al.*, 1989). At position 3305 a cytosine (wild type) was replaced by a thymine (variant), resulting in the replacement of amino acid 101 of VP-1 (Fig. 6). The parental DA strain contained at this position a threonine which was replaced by an isoleucine in the variant virus VP-1. Sequence alignment with the known structure of Mengo virus suggested that this amino acid replacement was located within an exposed loop on the surface of the virion, highly accessible to antibodies and at the periphery of a deep depression, proposed as a receptor-binding site (Pevear *et al.*, 1988b). The above results from ELISAs, neutralization assays, and RNA sequencing provided an opportunity to determine precisely structures important for neurovirulence.

VI. Viral Infectious Complementary DNA

In addition to sequence comparisons to determine important areas within the genome, studies using infectious complementary DNA clones have become very powerful. Infectious transcripts for different picornaviruses have been prepared: polioviruses (Racaniello and Baltimore, 1981; Omata *et al.*, 1984; Kaplan *et al.*, 1985), human rhinovirus type 14 (Mizutani and Colonno, 1985), coxsackie B3 virus (Kandolf and Hofschneider, 1985), EMCV (Naviaux *et al.*, 1990), hepatitis A (Cohen *et al.*, 1987), and foot-and-mouth disease virus (Zibert *et al.*, 1990). Infectious transcripts for members of both TMEV subgroups DA (Roos *et al.*, 1989b), BeAn (Calenoff *et al.*, 1990), and the highly virulent GDVII (Tangy *et al.*, 1989) have been constructed. These infectious transcripts allowed recombinant studies between the highly

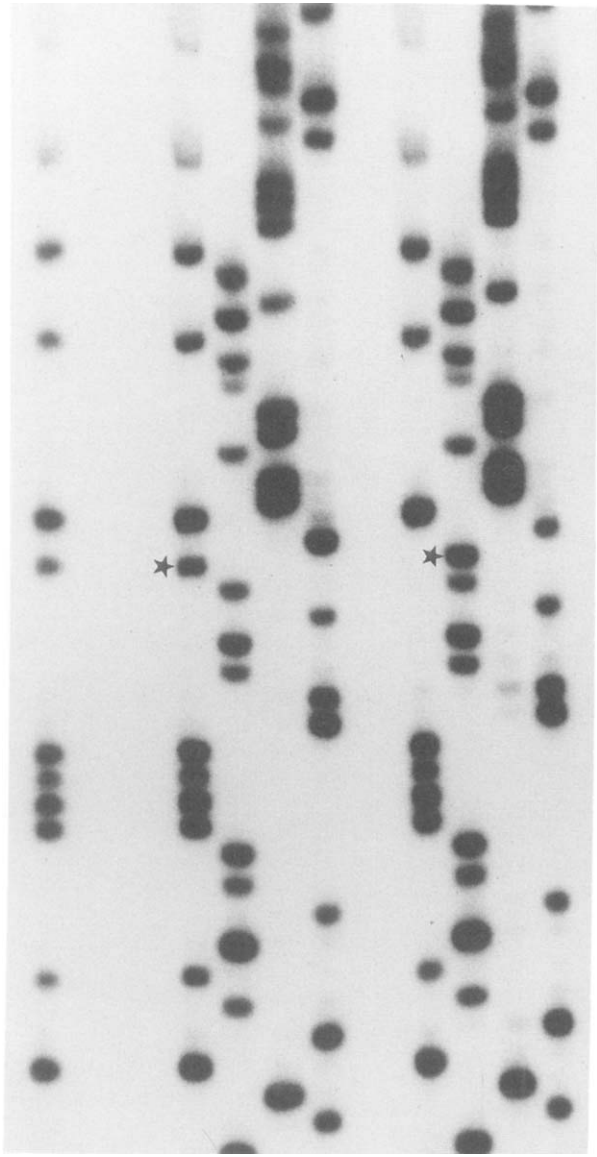

FIG. 6. Sequence comparisons between the parental DA strain (right panel) and the H7A6-2 virus variant demonstrate one single point mutation at position 3305. DA contains a cytosine (*) which is replaced in the variant virus by a thymine (*).

virulent and the less virulent TMEV strains. Using recombinant chimeras of GDVII and BeAn virus the mouse neurovirulence of TMEV was mapped to the region encoding the leader and coat proteins (Calenoff *et al.*, 1990). Thus specific areas can now be associated with viral functions and altered pathogenesis.

VII. Conclusions

Theiler's murine encephalomyelitis virus infection can cause a characteristic CNS disease (acute polioencephalomyelitis and chronic demyelinating disease) in susceptible mice. The chronic demyelinating disease serves as a model for human demyelinating disorders, and the pathogenesis of demyelination is under intense investigation. Data obtained from pathological and immunological analyses indicate that immune-mediated as well as direct viral injuries of myelin and/or oligodendrocytes contribute to the demyelination. Modern molecular biology techniques using infectious cDNAs will allow exact mapping of single epitopes by insertion of single point mutations in specific areas of interest. Exact knowledge of the epitopes responsible for a particular disease phenotype will allow a better understanding of virus-induced mechanisms which lead to disease.

Acknowledgments

The authors would like to thank Peggy Farness, Susan McClanahan, and Pamela Lewis for their excellent technical skills. This work was supported by National Multiple Sclerosis Society, GR1780 and GR2087, and National Institutes of Health, NS23162. R.S.F. is a Javits Neuroscience scholar.

References

Acharaya, R., Fry, E., Stuart, D., Fox, G., Rowlands, D., and Brown, F. (1989). The three-dimensional structure of foot-and-mouth disease virus at 2.9 Å resolution. *Nature (London)* **337,** 709–716.

Aubert, C., Chamorro, M., and Brahic, M. (1987). Identification of Theiler's virus infected cells in the central nervous system of the mouse during demyelinating disease. *Microb. Pathog.* **3,** 319–326.

Barbano, R. L., and Dal Canto, M. C. (1984). Serum and cells from Theiler's virus-infected mice fail to injure myelinating cultures or to produce *in vivo* transfer of disease. The pathogenesis of Theiler's virus-induced demyelination appears to differ from that of EAE. *J. Neurol. Sci.* **66,** 283–293.

Blakemore, W. F., Welsh, C. J. R., Tonks, P., and Nash, A. A. (1988). Observations on demyelinating lesions induced by Theiler's virus in CBA mice. *Acta Neuropathol.* **76,** 581–589.

Brahic, M., Stroop, W. G., and Baringer, J. R. (1981). Theiler's virus persists in glial cells during demyelinating disease. *Cell* **26,** 123–128.

Brahic, M., Haase, A. T., and Cash, E. (1984). Simultaneous *in situ* detection of viral RNA and antigens. *Proc. Natl. Acad. Sci. U.S.A.* **81,** 5445–5448.

Burns, C. C., Lawson, M. A., Semler, B. L., and Ehrenfeld, E. (1989). Effects of mutations in poliovirus $3D^{pol}$ on RNA polymerase activity and on polyprotein cleavage. *J. Virol.* **63,** 4866–4874.

Calenoff, M. A., Faaberg, K. S., and Lipton, H. L. (1990). Genomic regions of neurovirulence and attenuation in Theiler murine encephalomyelitis virus. *Proc. Natl. Acad. Sci. U.S.A.* **87,** 978–982.

Carrol, A. R., Rowlands, D. J., and Clarke, B. E. (1984). The complete nucleotide sequence of the RNA coding for the primary translation product of foot-and-mouth disease virus. *Nucleic Acids Res.* **12,** 2461–2472.

Cash, E., Bandeira, A., Chirinian, S., and Brahic, M. (1989). Characterization of B lymphocytes present in the demyelinating lesions induced by Theiler's virus. *J. Immunol.* **143,** 984–988.

Chamorro, M., Aubert, C., and Brahic, M. (1986). Demyelinating lesions due to Theiler's virus are associated with ongoing central nervous system infection. *J. Virol.* **57,** 992–997.

Clatch, R. J., Melvold, R. W., Miller, S. D., and Lipton, H. L. (1985). Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease in mice is influenced by the H-2D region, correlation with TMEV-specific delayed-type hypersensitivity. *J. Immunol.* **135,** 1408–1414.

Clatch, R. J., Lipton, H. L., and Miller, S. D. (1986). Characterization of Theiler's murine encephalomyelitis virus (TMEV)-specific delayed-type hypersensitivity response in TMEV-induced demyelinating disease, correlation with clinical signs. *J. Immunol.* **136,** 920–927.

Clatch, R. J., Melvold, R. W., Dal Canto, M. C., Miller, S. D., and Lipton, H. L. (1987a). The Theiler's murine encephalomyelitis virus (TMEV) model for multiple sclerosis shows a strong influence of the murine equivalent of HLA-A, B, and C. *J. Neuroimmunol.* **15,** 121–135.

Clatch, R. J., Lipton, H. L., and Miller, S. D. (1987b). Class II-restricted T cell responses in Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease. II. Survey of host immune responses and central nervous system virus titers in inbred mouse strains. *Microb. Pathog.* **3,** 327–337.

Clatch, R. J., Pevear, D. C., Rozhon, E., Roos, R. P., Miller, S. D., and Lipton, H. L. (1987c). Characterization and specificity of humoral immune responses to Theiler's murine encephalomyelitis virus capsid proteins. *J. Gen. Virol.* **68,** 3191–3196.

Cohen, J. I., Ticehurst, J. R., and Feinstone, S. M. (1987). Hepatitis A virus cDNA and its RNA transcripts are infectious in cell culture. *J. Virol.* **61,** 3035–3039.

Dal Canto, M. C. (1982). Uncoupled relationship between demyelination and primary infection of myelinating cells in Theiler's virus encephalomyelitis. *Infect. Immun.* **35,** 1133–1138.

Dal Canto, M. C., and Barbano, R. L. (1984). Remyelination during remission in Theiler's virus infection. *Am. J. Pathol.* **116,** 30–45.

Dal Canto, M. C., and Lipton, H. L. (1975). Primary demyelination in Theiler's virus infection. An ultrastructural study. *Lab. Invest.* **33,** 626–637.

Dal Canto, M., and Lipton, H. L. (1979). Recurrent demyelination in chronic central nervous system infection produced by Theiler's murine encephalomyelitis virus. *J. Neurol. Sci.* **42,** 391–405.

Dal Canto, M. C., and Lipton, H. L. (1980). Schwann cell remyelination and recurrent

demyelination in the central nervous system of mice infected with attenuated Theiler's virus. *Am. J. Pathol.* **98,** 101–122.

Dal Canto, M. C., and Lipton, H. L. (1982). Ultrastructual immunohistochemical localization of virus in acute and chronic demyelinating Theiler's virus infection. *Am. J. Pathol.* **106,** 20–29.

Dalziel, R. G., Lampert, P. W., Talbot, P. J., and Buchmeier, M. J. (1986). Site-specific alteration of murine hepatitis virus type 4 peplomer glycoprotein E2 results in reduced neurovirulence. *J. Virol.* **59,** 463–471.

Daniels, J. B., Pappenheimer, A. M., and Richardson, S. (1952). Observations on encephalomyelitis of mice (DA strain). *J. Exp. Med.* **96,** 517–530.

Dean, D. J. (1951). Mouse encephalomyelitis. Immunologic studies of a non-infected colony. *J. Immunol.* **66,** 347–359.

Fleming, J. O., Trousdale, M. D., El-Zaatari, F. A. K., Stohlman, S. A., and Weiner, L. P. (1986). Pathogenicity of antigenic variants of murine coronavirus JHM selected with monoclonal antibodies. *J. Virol.* **58,** 869–875.

Frankel, G., Friedmann, A., Amir, A., David, Y., and Shahar, A. (1986). Theiler's virus replication in isolated Schwann cell culture. *J. Neurosci. Res.* **15,** 127–136.

Friedmann, A., Frankel, G., Lorch, Y., and Steinman, L. (1987). Monoclonal anti-I-A antibody reverses chronic paralysis and demyelination in Theiler's virus-infected mice, critical importance of timing of treatment. *J. Virol.* **61,** 898–903.

Fujinami, R. S., and Zurbriggen, A. (1989). Is Theiler's murine encephalomyelitis virus infection of mice an autoimmune disease? *APMIS* **97,** 1–8.

Fujinami, R. S., Zurbriggen, A., and Powell, H. C. (1988). Monoclonal antibody defines determinant between Theiler's virus and lipid-like structures. *J. Neuroimmunol.* **20,** 25–32.

Fujinami, R. S., Rosenthal, A., Lampert, P. W., Zurbriggen, A., and Yamada, M. (1989). Survival of athymic (nu/nu) mice after Theiler's murine encephalomyelitis virus infection by passive administration of neutralizing monoclonal antibody. *J. Virol.* **63,** 2081–2087.

Graves, M. C., Bologa, L., Siegel, L., and Londe, H. (1986). Theiler's virus in brain cell culture: Lysis of neurons and oligodendrocytes and persistence in astrocytes and macrophages. *J. Neurosci. Res.* **15,** 491–501.

Hogle, J. M., Chow, M., and Filman, D. J. (1985). Three-dimensional structure of poliovirus at 2.9 Å resolution. *Science* **229,** 1358–1365.

Hogle, J. M., Chow, M., and Filman, D. J. (1987). The structure of poliovirus. *Sci. Am.* **255,** 42–49.

Kandolf, R., and Hofschneider, P. H. (1985). Molecular cloning of the genome of a cardiotropic Coxsackie B3 virus, full-length reverse-transcribed recombinant cDNA generates infectious virus in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 4818–4822.

Kaplan, G., Lubinski, J., Dasgupta, A., and Racaniello, V. R. (1985). *In vitro* synthesis of infectious poliovirus RNA. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 8424–8428.

Lang, W., Wiley, C., and Lampert, P. (1985). Theiler's virus encephalomyelitis is unaffected by treatment with myelin components. *J. Neuroimmunol.* **9,** 109–113.

Larsen, G. R., Dorner, A. J., Harris, T. J. R., and Wimmer, E. (1980). The structure of poliovirus replicative form. *Nucleic Acids Res.* **8,** 1217–1229.

Lehrich, J. R., Arnason, B. G., and Hochberg, F. H. (1976). Demyelinating myelopathy in mice induced by the DA virus. *J. Neurol. Sci.* **29,** 149–160.

Lindsley, M. D., and Rodriguez, M. (1989). Characterization of the inflammatory response in the central nervous system of mice susceptible or resistant to demyelination by Theiler's virus. *J. Immunol.* **142,** 2677–2682.

Lindsley, M. D., Thiemann, R. L., and Rodriguez, M. (1988). Expression of Class I antigen in C57/BL10 and SJL mice following Theiler's virus infection. *J. Neuropathol. Exp. Neurol.* **47,** 324.

Lipton, H. L. (1975). Theiler's virus infection in mice, an unusual biphasic disease process leading to demyelination. *Infect. Immun.* **11,** 1147–1155.

Lipton, H. L. (1978). Characterization of the TO strains of Theiler's mouse encephalomyelitis viruses. *Infect. Immun.* **20,** 869–872.

Lipton, H. L., and Dal Canto, M. C. (1976a). Chronic neurologic disease in Theiler's virus infection of SJL/J mice. *J. Neurol. Sci.* **30,** 201–207.

Lipton, H. L., and Dal Canto, M. (1976b). Theiler's virus-induced demyelination, prevention by immunosuppression. *Science* **192,** 62–64.

Lipton, H. L., and Dal Canto, M. C. (1977). Contrasting effects of immunosuppression on Theiler's virus infection in mice. *Infect. Immun.* **15,** 903–909.

Lipton, H. L., and Dal Canto, M. C. (1979). Susceptibility of inbred mice to chronic central nervous system infection by Theiler's murine encephalomyelitis virus. *Infect. Immun.* **26,** 369–374.

Lipton, H. L., and Friedmann, A. (1980). Purification of Theiler's murine encephalomyelitis virus and analysis of the structural virion polypeptides, correlation of the polypeptide profile with virulence. *J. Virol.* **33,** 1165–1172.

Lipton, H. L., and Gonzalez-Scarano, F. (1978). Central nervous system immunity in mice infected with Theiler's virus. I. Local neutralizing antibody response. *J. Infect. Dis.* **137,** 145–151.

Lipton, H. L., and Melvold, R. (1984). Genetic analysis of susceptibility to Theiler's virus-induced demyelinating disease in mice. *J. Immunol.* **132,** 1821–1825.

Lipton, H. L., Miller, S., Melvold, R., and Fujinami, R. S. (1986). Theiler's murine encephalomyelitis virus (TMEV) infection in mice as a model for multiple sclerosis. *In* "Concepts in Viral Pathogenesis II" (A. L. Notkins and M. B. A. Oldstone, eds.), pp. 248–254. Springer-Verlag, New York.

Lloyd, R. E., Grubman, M. J., and Ehrenfeld, E. (1988). Relationship of p220 cleavage during picornavirus infection to 2A proteinase sequencing. *J. Virol.* **62,** 4216–4223.

Lorch, Y., Kotler, M., and Friedmann, A. (1984). Persistent and acute central nervous system infections are caused by Theiler's murine encephalomyelitis viruses which differ in RNA composition but code for only slightly different proteins. *J. Virol.* **52,** 960–965.

Love, S. (1987). Distribution of Theiler's virus in the CNS of athymic nude mice, effect of varying the route of inoculation. *J. Neurol. Sci.* **81,** 55–66.

Luo, M., Vriend, G., Kamer, G., Minor, I., Arnold, E., Rossmann, M. G., Boege, U., Scraba, D. G., Duke, G. M., and Palmenberg, A. C. (1987). The atomic structure of Mengo virus at 3.0 Å resolution. *Science* **235,** 182–191.

Melnick, J. L., and Riordan, J. T. (1947). Latent mouse encephalomyelitis. *J. Immunol.* **57,** 331–342.

Melvold, R. W., Jokinen, D. M., Knobler, R. L., and Lipton, H. L. (1987). Variations in genetic control of susceptibility to Theiler's murine cencephalomyelitis virus (TMEV)-induced demyelinating disease. I. Differences between susceptible SJL/J and resistant BALB/C strains map near the T cell beta-chain constant genes on chromosome 6. *J. Immunol.* **138,** 1429–1433.

Miller, S. D., Clatch, R. J., Pevear, D. C., Trotter, J. L., and Lipton, H. L. (1987). Class II-restricted T cell responses in Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease. I. Cross-specificity among TMEV substrains and related picornaviruses, but not myelin proteins. *J. Immunol.* **138,** 3776–3784.

Mizutani, S., and Colonno, R. J. (1985). *In vitro* synthesis of an infectious RNA from cDNA clones of human rhinovirus type 14. *J. Virol.* **56,** 628–632.

Naviaux, R. K., Cohen, S. H., Vanden Brink, K. M., and Jordan, G. W. (1990). Construction and characterization of two infectious clones of encephalomyocarditis virus. *J. Virol.* **64,** 913–917.

Nitayaphan, S., Toth, M. M., and Roos, R. P. (1985a). Neutralizing monoclonal antibodies to Theiler's murine encephalomyelitis viruses. *J. Virol.* **53,** 651–657.

Nitayaphan, S., Toth, M. M., and Roos, R. P. (1985b). Localization of a neutralization site of Theiler's murine encephalomyelitis viruses. *J. Virol.* **56,** 887–895.

Nitayaphan, S., Omilianowski, D., Toth, M. M., Parks, G. D., Rueckert, R. R., Palmenberg, A. C., and Roos, R. P. (1986). Relationship of Theiler's murine encephalomyelitis viruses to the cardiovirus genus of picornaviruses. *Intervirology* **26,** 140–148.

Ohara, Y., and Roos, R. (1987). The antibody response in Theiler's virus infection, new perspectives on multiple sclerosis. *Prog. Med. Virol.* **34,** 156–179.

Ohara, Y., Stein, S., Fu, J., Stillman, L., Klaman, R., and Roos, P. (1988). Molecular cloning and sequence determination of DA strain of Theiler's murine encephalomyelitis viruses. *Virology* **164,** 245–255.

Olitsky, P. K. (1940). A transmissible agent (Theiler's virus) in the intestine of normal mice. *J. Exp. Med.* **72,** 113–127.

Olitsky, P. K. (1945). Certain properties of Theiler's virus, especially in relation to its use as model for poliomyelitis. *Proc. Soc. Exp. Biol. Med.* **58,** 77–81.

Olitsky, P. K., and Schlesinger, R. W. (1941). Histopathology of CNS of mice infected with virus of Theiler's disease (spontaneous encephalomyelitis). *Proc. Soc. Exp. Biol. Med.* **47,** 79–83.

Omata, T., Kohara, M., Sakai, Y., Kameda, A., Imura, N., and Nomoto, A. (1984). Cloned infectious complementary DNA of the poliovirus Sabin 1 genome, biochemical and biological properties of the recovered virus. *Gene* **32,** 1–10.

O'Neill, R. E., and Racaniello, V. R. (1989). Inhibition of translation in cells infected with a poliovirus $2A^{pro}$ mutant correlates with phosphorylation of the alpha subunit of eucaryotic initiation factor 2. *J. Virol.* **63,** 5069–5075.

Ozden, S., Tangy, F., Chamorro, M., and Brahic, M. (1986). Theiler's virus genome is closely related to that of encephalomyocarditis virus, the prototype cardiovirus. *J. Virol.* **60,** 1163–1165.

Ozden, S., Aubert, C., and Brahic, M. (1988). Expression of Theiler's murine encephalomyelitis virus protease 3C and polymerase 3D in *Escherichia coli* and characterization of monospecific sera. *Virology* **165,** 596–600.

Page, G. S., Mosser, A. G., Hogle, J. M., Filman, D. J., Rueckert, R. R., and Chow, M. (1988). Three-dimensional structure of poliovirus serotype 1 neutralizing determinants. *J. Virol.* **62,** 1781–1794.

Parker, R. C., and Hollender, A. J. (1945). Propagation of Theiler's GDVII mouse virus in tissue culture. *Proc. Soc. Exp. Biol. Med.* **60,** 88–93.

Paya, C. V., Patick, A. K., Leibson, P. J., and Rodriguez, M. (1989). Role of natural killer cells as immune effectors in encephalitis and demyelination induced by Theiler's virus. *J. Immunol.* **143,** 95–102.

Penney, J. B., and Wolinsky, J. S. (1979). Neuronal and oligodendroglial infection by the WW strain of Theiler's virus. *Lab. Invest.* **40,** 324–330.

Pevear, D. C., Calenoff, M. A., and Lipton, H. L. (1987a). Sequence comparison of a highly virulent and a less virulent (demyelinating) strain of Theiler's murine encephalomyelitis virus. *J. Neuroimmunol.* **16,** 204.

Pevear, D. C., Calenoff, M. A., Rozhon, E., and Lipton, H. L. (1987b). Analysis of the

complete nucleotide sequence of the picornavirus Theiler's murine encephalomyelitis virus indicates that it is closely related to cardioviruses. *J. Virol.* **61,** 1507–1516.

Pevear, D. C., Borkowsky, J., Calenoff, M. A., Oh, C. K., Ostrowski, B., and Lipton, H. L. (1988a). Insights into Theiler's virus neurovirulence based on a genomic comparison of the neurovirulent GDVII and less virulent BeAn strains. *Virology* **165,** 1–12.

Pevear, D. C., Luo, M., and Lipton, H. L. (1988b). Three-dimensional model of the capsid proteins of two biologically different Theiler virus strains, clustering of amino acid differences identifies possible locations of immunogenic sites on the virion. *Proc. Natl. Acad. Sci. U.S.A.* **85,** 4496–4500.

Powell, H. C., Lehrich, J. R., and Arnason, B. G. (1977). Electron-microscopic appearance of the DA virus, a demyelinating murine virus. *J. Neurol. Sci.* **34,** 15–23.

Rabinowitz, S. G., and Lipton, H. L. (1976). Cellular immunity in chronic Theiler's virus central nervous system infection. *J. Immunol.* **117,** 357–363.

Racaniello, V. R., and Baltimore, D. (1981). Cloned poliovirus complementary DNA is infectious in mammalian cells. *Science* **214,** 916–919.

Rauch, H. C., and Montgomery, I. N. (1988). The role of the immune response in TMEV infection and the development of late onset demyelination. *J. Immunol.* **136,** 2136–2140.

Rauch, H. C., Montgomery, I. N., Hinman, C. L., Harb, W., and Benjamins, J. A. (1987). Chronic Theiler's virus infection in mice, appearance of myelin basic protein in the cerebrospinal fluid and serum antibody directed against MBP. *J. Neuroimmunol.* **14,** 35–48.

Rodriguez, M. (1985). Virus-induced demyelination in mice, "dying back" of oligodendrocytes. *Mayo Clin. Proc.* **60,** 433–438.

Rodriguez, M., and David, C. S. (1985). Demyelination induced by Theiler's virus, influence of the H-2 haplotype. *J. Immunol.* **135,** 2145–2148.

Rodriguez, M., and Sriram, S. (1988). Successful therapy of Theiler's virus-induced demyelination (DA strain) with monoclonal anti-Lyt-2 antibody. *J. Immunol.* **140,** 2950–2955.

Rodriguez, M., Leibowitz, J. L., Powell, H. C., and Lampert, P. W. (1983a). Neonatal infection with the Daniels strain of Theiler's murine encephalomyelitis virus. *Lab. Invest.* **49,** 672–679.

Rodriguez, M., Leibowitz, J. L., and Lampert, P. W. (1983b). Persistent infection of oligodendrocytes in Theiler's virus-induced encephalomyelitis. *Ann. Neurol.* **13,** 426–433.

Rodriguez, M., Leibowitz, J., and David, C. S. (1986a). Susceptibility to Theiler's virus-induced demyelination. Mapping of the gene within the H-2D region. *J. Exp. Med.* **163,** 620–631.

Rodriguez, M., Lafuse, W. P., Leibowitz, J., and David, C. S. (1986b). Partial suppression of Theiler's virus-induced demyelination *in vivo* by administration of monoclonal antibodies to immune-response gene products. *Neurology* **36,** 964–970.

Rodriguez, M., Oleszak, E., and Leibowitz, J. (1987a). Theiler's murine encephalomyelitis, a model of demyelination and persistence of virus. *CRC Crit. Rev. Immunol.* **7,** 325–365.

Rodriguez, M., Peirce, M. L., and Howie, E. A. (1987b). Immune response gene products (Ia antigen) on glial and endothelial cells in virus-induced demyelination. *J. Immunol.* **138,** 3438–3442.

Rodriguez, M., Lucchinetti, C. F., Clark, R. J., Yaksh, T. L., Markovitz, H., and Lennon, V. A. (1988). Immunoglobulins and complement in demyelination induced in mice by Theiler's virus. *J. Immunol.* **140,** 800–806.

Roos, R. P., and Wollmann, R. (1984). DA strain of Theiler's murine encephalomyelitis virus induces demyelination in nude mice. *Ann. Neurol.* **15,** 494–499.

Roos, R. P., Firestone, S., Wollmann, R., Variakojis, D., and Arnason, B. G. W. (1982). The effect of short-term and chronic immunosuppression on Theiler's virus demyelination. *J. Neuroimmunol.* **2,** 223–234.

Roos, R. P., Nalefski, E. A., Nitayaphan, S., Variakojis, R., and Singh, K. K. (1987). An isoelectric focusing overlay study of the humoral immune response in Theiler's virus demyelinating disease. *J. Neuroimmunol.* **13,** 305–314.

Roos, R. P., Kong, W.-P., and Semler, B. L. (1989a). Polyprotein processing of Theiler's murine encephalomyelitis virus. *J. Virol.* **63,** 5344–5353.

Roos, R. P., Stein, S., Ohara, Y., Fu, J., and Semler, B. L. (1989b). Infectious cDNA clones of the DA strain of Theiler's murine encephalomyelitis virus. *J. Virol.* **63,** 5492–5496.

Roos, R. P., Stein, S., Routbort, M., Senkowski, A., Bodwell, T., and Wollmann, R. (1989c). Theiler's murine encephalomyelitis virus neutralization escape mutants have a change in disease phenotype. *J. Virol.* **63,** 4469–4473.

Rosenthal, A., Fujinami, R. S., and Lampert, P. W. (1986). Mechanism of Theiler's virus-induced demyelination in nude mice. *Lab. Invest.* **54,** 515–522.

Rozhon, E. J., Lipton, H. L., and Brown, F. (1982). Characterization of Theiler's murine encephalomyelitis virus RNA. *J. Gen. Virol.* **61,** 157–165.

Rubio, N., and Cuesta, A. (1989). Lack of cross-reaction between myelin basic proteins and putative demyelinating virus envelope proteins. *Mol. Immunol.* **26,** 663–668.

Rueckert, R. R. (1990). Picornaviridae and their replication. *In* "Virology" (B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath, and B. Roizman, eds.), pp. 507–548. Raven, New York.

Skern, T., Sommergruber, W., Blaas, D., Gruendler, P., Fraundorfer, F., Pieler, C. H., Fogy, I., and Kuechler, E. (1985). Human rhinovirus 2, complete nucleotide sequence and proteolytic processing signals in the capsid protein region. *Nucleic Acids Res.* **8,** 2111–2126.

Spriggs, D. R., and Fields, B. N. (1982). Attenuated reovirus type 3 strains generated by selection of haemagglutinin antigenic variants. *Nature (London)* **297,** 68–70.

Stroop, W. G., and Baringer, J. R. (1981). Biochemistry of Theiler's murine encephalomyelitis virus isolated from acutely infected mouse brain: Identification of a previously unreported polypeptide. *Infect. Immun.* **32,** 769–777.

Stroop, W. G., Baringer, J. R., and Brahic, M. (1981). Detection of Theiler's virus RNA in mouse central nervous system by *in situ* hybridization. *Lab. Invest.* **45,** 504–509.

Stroop, W. G., Brahic, M., and Baringer, J. R. (1982). Detection of tissue culture-adapted Theiler's virus RNA in spinal cord white matter cells throughout infection. *Infect. Immun.* **37,** 763–770.

Takahara, Y., Ando, N., Kohara, M., Hagiono-Yamagishi, K., Nomoto, A., Itho, H., Numao, N., and Kondo, K. (1989). Purification of enzymatically active poliovirus proteinase 3C produced in *Escherichia coli*. *Gene* **79,** 249–258.

Tangy, F., McAllister, A., and Brahic, M. (1989). Molecular cloning of the complete genome of strain GDVII of Theiler's virus and production of infectious transcripts. *J. Virol.* **63,** 1101–1106.

Theiler, M. (1934). Spontaneous encephalomyelitis of mice. A new virus disease. *Science* **80,** 122.

Theiler, M. (1937). Spontaneous encephalomyelitis of mice. A new virus disease. *J. Exp. Med.* **65,** 705–719.

Theiler, M., and Gard, S. (1940a). Encephalomyelitis of mice. I. Characterization and pathogenesis of the virus. *J. Exp. Med.* **72,** 49–67.

Theiler, M., and Gard, S. (1940b). Encephalitis of mice. III. Epidemiology. *J. Exp. Med.* **72,** 79–90.

von Magnus, H. (1951). Studies on mouse encephalomyelitis virus (TO strain). III. Spread and multiplication of TO virus in baby mice after various routes of inoculation. *Acta Pathol. Microbiol. Scand.* **28,** 234–249.

Welsh, C. J., Tonks, P., Nash, A. A., and Blakemore, W. F. (1987). The effect of L3T4 T cell depletion on the pathogenesis of Theiler's murine encephalomyelitis virus infection in CBA mice. *J. Gen. Virol.* **68,** 1659.

Wimmer, E. (1979). The genome-linked protein of picornavirus: Discovery, properties, and possible functions. *In* "The Molecular Biology of Picornaviruses" (R. Perez-Bercoff, ed.), pp. 175–190. Plenum, New York.

Wroblewska, Z., Gilden, D. H., Wellish, M., Rorke, L. B., Warren, K. G., and Wolinsky, J. S. (1977). Virus-specific intracytoplasmic inclusions in mouse brain produced by a newly isolated strain of Theiler virus. I. Virologic and morphologic studies. *Lab. Invest.* **37,** 595–602.

Wroblewska, Z., Kim, S. U., Sheffield, W. D., and Gilden, D. H. (1979). Growth of the WW strain of Theiler virus in mouse central nervous system organotypic culture. *Acta Neuropathol.* **47,** 13–19.

Yamada, M., Zurbriggen, A., and Fujinami, R. S. (1990a). Monoclonal antibody to Theiler's virus defines a determinant on myelin and oligodendrocytes, and augments demyelination in experimental allergic encephalomyelitis. *J. Exp. Med.* **171,** 1893–1907.

Yamada, M., Zurbriggen, A., and Fujinami, R. S. (1990b). The relationship between viral RNA, myelin-specific mRNAs, and demyelination in the central nervous system disease during Theiler's virus infection. *Am. J. Path.* (in press).

Zibert, A., Maass, S., Strebel, K., Falk, M. M., and Beck, E. (1990). Infectious foot-and-mouth disease virus derived from a cloned full-length cDNA. *J. Virol.* **64,** 2467–2473.

Zurbriggen, A., and Fujinami, R. S. (1988). Theiler's virus infection in nude mice: viral RNA in vascular endothelial cells. *J. Virol.* **62,** 3589–3596.

Zurbriggen, A., and Fujinami, R. S. (1989). A neutralization-resistant Theiler's virus variant produces an altered disease pattern in the mouse central nervous system. *J. Virol.* **63,** 1505–1513.

Zurbriggen, A., Hogle, J. M., and Fujinami, R. S. (1989). Alteration of amino acid 101 within capsid protein VP-1 changes the pathogenicity of Theiler's murine encephalomyelitis virus. *J. Exp. Med.* **170,** 2037–2049.