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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
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 non-enveloped 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 protease 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 Friedman, 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

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**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 (poliomyelitis) 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).
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.
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. 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.
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 CSH/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,
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 immuno-
nopathological 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 infec-
tion 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 propa-
gated in BHK-21 (baby hamster kidney) cell and L2 cell cultures.
Cellular pathology after infection with TMEV in vitro has been de-
scribed (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) ob-
served 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 in-
cclusions containing viral paracrystalline arrays in astrocytes. Graves
et al. (1986) found, in primary brain cell cultures derived from new-
born 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-pro-
ducing 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 cytoto-
ic T cell immune response to infected brain cells may lead to per-
sistence of virus infection. Impairment of suppressor T cell function
may allow damage to myelin or oligodendrocytes mediated by class II-
restricted \([CD4^+ \text{ (helper or delayed hypersensitivity type)}]\) T cells.
Another gene related to susceptibility for TMEV-induced demyelinating
disease is closely linked to a gene encoding the \(\beta\) 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-re-
stricted 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 immu-
nosuppression by cyclophosphamide or anti-thymocyte serum de-
creased demyelination induced by TMEV, suggesting an immune-
mediated mechanism of demyelination. In those mice, both the humor-
al and cellular immune responses to the virus were suppressed. Later
Roos et al. (1982) demonstrated that only a short-term immunosup-
pressive 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 hyper-
sensitivity (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-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 re-
results suggest that demyelination is mediated by CD8\(^+\) (cytotoxic/sup-
pressor), 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-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-2\(^+\) (CD8\(^+\)) cells during demyelination, and parenchymal distribution of Lyt-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 de-
myelinating disease in naive recipients, although transfer of the virus
could not be ruled out.

Combined with the results of the immunogenetic analysis and im-
munosuppression 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). Cere-
brosplai 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 iso-
lated 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., 1987~) (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 mye-
lin components have been investigated to test a possibility of autoim-
mune 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 \textit{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 \textit{et al.}, 1988). MAb H8 reacted with oligodendrocytes in brain cell cultures and myelin structures in frozen brain sections (Yamada \textit{et al.}, 1990a). When injected into mice with EAE, MAb H8 increased the demyelination by 10-fold (Yamada \textit{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 \textit{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 \textit{et al.}, 1988; Yamada \textit{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.

\textit{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 \textit{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 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 INFECTIONOUS 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.

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